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Howard H. Sky-Peck
Department of Biochemistry
Rush Presbyterian-St. Luke's Medical Center
Chicago, IL 60612 (USA)

ATTACHMENT C-2

Review of

*Manganese, a Physiological Marker for Criminal Violence* by Gottschalk et al. (undated)

Emanuel Landau, Ph.D.

A review of the Gottschalk et al. undated study justifiably would conclude that the use of "convenience" samples, the apparent picking and choosing the one toxic metal out of 23 that seem to distinguish the criminal population, and the failure to adjust for factors other than incarceration in comparing violent criminals with control populations make this a scientifically unacceptable report.

This study was designed to test the hypothesis that "the pathogenesis of violence possibly can be traced through hair analysis." However, the authors appear to recognize that there are two steps to validate in this hypothesis. First, it must be demonstrated that "selected potentially toxic metals and aberrant behavior" are correlated, and second, the mechanism by which mild manganese toxicity precipitates violent behavior must be detailed. Obviously, this second step cannot be addressed with any scientific acceptability in the study as conducted.

In their 1987 cross-sectional epidemiological study of workers exposed to inorganic manganese in a manganese oxide and salt producing plant, Roels et al. noted the absence of good biological indicators of manganese exposure. There was some indication that "on a group basis Mn in urine reflects mainly current exposure, whereas Mn in blood seems more influenced by the body burden of Mn." The study by Gottschalk et al. uses hair as a biological indicator because of its ease of collection and analysis and the concentration of trace elements at levels higher than in blood or urine, thus providing a record of past toxic mineral status. Occupational health studies have used hair and nails in the past as biological indicators in addition to blood and urine for selected heavy metals. For example, see "Peripheral Neuropathy in Arsenic Smelter Workers" by Feldman et

al. (Neurology: 29:939-944, July 1979). This was a double-blind controlled study of individuals exposed to arsenic trioxide in a copper smelter factory. The workers were necessarily exposed to other metals, particularly copper, but also lead, cadmium, nickel, and zinc. It is of interest that the Gottschalk et al. study was originally designed (Stage 1) to obtain lead, cadmium, and copper levels in hair because these had previously been reported "to be associated with violent behavior (Schauss, 1981)."

The Gottschalk study failed to replicate the finding of the Schauss study, but by examining the remaining 20 minerals managed to find one, manganese, which seemed to fit. Statisticians, however, have learned to be wary of the process of data dredging and the consequent undue role of chance. Failure to recognize this, combined with the apparent lack of any statistical concern with the process of sample selection, seems to indicate that only after the data were collected and ready for analysis was statistical help obtained.

According to the text (page 4), the 104 male prisoners were "randomly" selected from those imprisoned for at least a year prior to selection. Approximately one-third of this "random sample" were Caucasian, one-third were Hispanic, and the remainder were Black. Controls were matched for "age, sex, and race" (page 5). No information is provided for the process of age matching. Mean age is used instead of the more useful median used in demographic research. This reviewer is unable to find acceptable a process of age matching which could result in a mean age of 29 years for the violent prisoners versus a mean age of 24 years for the town controls and a mean age of 31 years for the guards. The matching by race is wholly incomprehensible. How do 39 Caucasian, 33 Hispanic, and 32 Black prisoners get matched by 62 Caucasians, 10 Hispanics, and 11 Blacks? One may ask at this juncture: Are manganese hair levels differentially found in the hair of the three races listed? There is some indication of such difference in the statement on page 10 about normal population values for Caucasian males. Also, Phase III of the study was limited to Caucasian males. Incidentally, isn't it possible that

the higher levels of lead in the hair of prison guards (page 7) is related to the use of firing ranges where airborne levels of lead may be very substantial?

The sample selection for Phase II involved 60 male inmates "recruited" from those awaiting trial for violent crimes. They were equally divided by race: one-third Caucasian, one-third Hispanic, and one-third Black. The male controls were all "recruited" from nearby barbershops and were stated to be matched for age, sex, and race. There were no longer any guards used as controls. Yet, 10 Caucasians, 10 Hispanics, and 10 Blacks do not add up to the 42 controls (page 5 and Table 1). The mean age of the prisoners, 27 years, may be significantly different from the 22 years for the barbershop users. Phase III was limited to male Caucasian prisoners and controls. Again, no guards were used as controls. The mean ages appear to be significantly different. An average age of 27 years for prisoners and 33 years for controls does not appear to represent acceptable matching procedures.

Although I have participated in occupational health studies involving biological indicators, I have never encountered Doctors Data Laboratories, Inc. Is this an authoritative reference for "normal" values of toxic metals, by race? Also, I am unable to understand the last sentence in the third paragraph of page 9. As presented, it is meaningless to me.

The concern for the careful collection of hair samples was not matched by the complete lack of care in obtaining information about the prisoners, the guards, or the other controls. The failure to obtain information on alcohol usage, smoking, medical and occupational histories, recent traumas, etc. is inexcusable in a scientific study. Incidentally, must hair samples be limited to those who have <u>never</u> used dyes, bleaches, or other treatments? Blinding the hair samples does not in and of itself insure a valid study. Also, use of relatively sophisticated tests of significance cannot compensate for major deficiencies in the statistical design of the study.

I am concerned with the apparent instability of the manganese levels in the hair of the prisoners. A mean level of 2.20 ppm in Phase I, followed by a mean manganese level of 1.39 in Phase II and 0.71 in Phase III is deeply disquieting. Given these sharp changes, the grouping together of these three studies seems to violate sound statistical practice. Again, shouldn't discriminant analysis have been used to separate the prisoners from the controls rather than relying on empirical observations? Note that for Phase III the mean manganese level was 0.71, thus barely meeting the criterion. Also, Schauss found elevated levels of manganese in his controls (0.74) rather than in his criminals (0.56).

Finally, are there any data which indicate that occupational exposure to manganese at levels much higher (2 to 6 times higher) than those seen in the prisoners (page 13) predisposes the workers to violent crimes? Is this of concern?

In summary, this is a scientifically unsound study. The use of "convenience" samples, the lack of proper matching, and the inability to understand the need for critical information about the population under study all serve to minimize the usefulness of the work. Blinding of hair samples and use of different statistical tests of significance cannot compensate for flawed study design.

ATTACHMENT C-3

Comments on:

*Manganese, a Physiological Marker for Criminal Violence*, by Louis A. Gottschalk, Tessio Rebello, Monte S. Buchsbaum, Howard G. Tucker, and E.L. Hodges. Unpublished draft.

By Ian Higgins, M.D.

Several studies have reported histories of head injuries, learning disabilities, or neurological problems in violent criminals. Neuropsychological tests have revealed evidence of brain dysfunction in a small proportion of violent offenders. Certain toxic metals may cause encephalopathy in humans and rats. An association between lead, cadmium, and copper in hair and violent behaviour was reported by Schauss (1981). The aim of Phase 1 of this research was to try to replicate this study. The objective of Phases 2 and 3 was to focus on a difference in hair manganese concentrations between violent criminals and non-criminal controls, which had been observed in Phase 1.

Hair samples were assayed for 23 metals by Doctors Data Laboratories, Inc. of West Chicago, Illinois. The samples were analysed by inductively coupled plasma atomic emission spectrophotometry. A standard hair sample was randomly added to each group of about 25 samples as a check on reliability. Investigators were blind to the origin of the samples until after the data had been tabulated.

In Phase 1, conducted in 1984, 104 male violent criminals who had been in jail for one year or more were compared with two groups of controls. The first group comprised men attending barbershops within a 50-mile radius of the jail; the second group comprised guards in the jail. The controls are said to have been matched for age, sex, and race to the felons, but Table 1 shows

that with the exception of sex -- all studies were confined to males -- this matching was not very successful. Thus there were 104 prisoners (39 Caucasian, 33 Hispanics, and 32 Blacks) with a mean age of 29 years; 52 town controls (42 Caucasians, 10 Hispanics, and no Blacks) with a mean age of 24 years; and 31 guards (20 Caucasians, no Hispanics, and 11 Blacks) with a mean age of 31 years.

The results of the hair comparisons are shown in Table 1 (all values in ppm).

| *Metal* | *Prisoners* | *Town Controls* | *Guards* |
|---|---|---|---|
| Lead | 12.1 ± 5.19 | 7.3 ± 1.01 | 13.3 ± 6.55?? |
| Cadmium | 0.48 ± 0.0062 | 0.71 ± 0.169 | 0.74 ± 0.161 |
| Copper | RESULTS NOT GIVEN | | |
| Manganese | 2.20 ± 0.24 | 0.30 ± 0.043 | 0.55 ± 0.128 |

The differences between the prisoners for lead, cadmium, and copper were not statistically significant, but the differences for manganese were highly significant. Thus the Schauss study was not confirmed, but a difference appeared for manganese. Phases 2 and 3 therefore focussed on this metal.

In Phase 2, conducted in 1987, 60 inmates (20 Caucasians, 20 Hispanics, and 20 Blacks) awaiting trial for violent crimes in Los Angeles and San Bernardino County jails were compared with 42 conviction-free subjects (10 Caucasians, 10 Hispanics, and 10 Blacks) from surrounding area barbershops.

[There appears to be an error in the total or the distribution of the controls.]

The mean manganese for the prisoners was 1.39 ± 0.297 ppm compared with 0.41 ± 0.070 ppm for the controls. The difference was statistically significant.

In Phase 3, conducted in 1988 at the San Bernardino County jail, male Caucasians who were charged with violent crimes were compared with 59 non-criminal male Caucasian subjects. Thirty of these controls came from the San Bernardino area, and 29 of them from Grange County located 50 miles away.

The mean hair sample of the prisoners was 0.71 ± 0.144 ppm; that of the controls was 0.33 ± 0.033 ppm. Again, the difference was statistically significant.

The authors comment that if 0.7 ppm was taken as a discriminant function test, 62% of the prisoners but only 10% of the controls were selected.

Comment:

There seems little doubt that in these studies the manganese concentration in hair of the prisoners was higher than that in the hair of the controls. This was so in all three phases of the study, conducted in 1984, 1987, and 1988. It was so in short-term as well as long-term criminals, and does not therefore appear to be due to any circumstances associated with incarceration. The difference was observed when comparisons were restricted to Caucasians. It cannot therefore be attributed to a higher manganese concentration in the hair of Black subjects. Although the matching with respect to age was not very good, age does not appear a likely explanation.

The main weakness of these studies is that none of the other probable reasons for a difference in hair manganese are considered. These differences include socioeconomic circumstances, diet, smoking, residence in more polluted areas, and occupational exposures. Further research on these topics would be interesting.

It may be significant that the manganese concentrations in the prisoners in the three phases has been declining. Just one more phase might show no significant difference.

*Prisoners*

| | |
|---|---|
| Phase 1 | 2.20 ± 0.24 |
| Phase 2 | 1.39 ± 0.297 |
| Phase 3 | 0.71 ± 0.144 |

# ATTACHMENT C-4

Stauber and Florence (1989)

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# MANGANESE IN SCALP HAIR: PROBLEMS OF EXOGENOUS MANGANESE AND IMPLICATIONS FOR MANGANESE MONITORING IN GROOTE EYLANDT ABORIGINES

J.L. STAUBER and T.M. FLORENCE

*CSIRO Centre for Advanced Analytical Chemistry, Division of Fuel Technology, Private Mail Bag 7, Menai NSW 2234 (Australia)*

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## ABSTRACT

The use of scalp hair to monitor manganese was studied as part of an investigation of manganese intoxication amongst a group of Aborigines living on manganese-rich soil on Groote Eylandt, in the Northern Territory of Australia. High scalp-hair manganese values were due largely to manganese from exogenous sources. Manganese (IV) dioxide in dust, trapped in hair, was reduced by the components of sweat, leading to the diffusion of manganese (II) into the hair shaft. At least 15 $\mu g$ Mn $g^{-1}$ hair could be incorporated into hair via this exogenous route.

To overcome the problems of manganese contamination, the ability of a number of leaching agents to remove exogenous manganese selectively from hair was tested. Measurements of manganese along the length of hair strands were extrapolated back to zero length to estimate the amount of manganese in the hair as it emerged from the scalp. Using this extrapolation technique, Aborigines on Groote Eylandt had a mean scalp-hair manganese of 16 ppm. Aborigines in non-manganese areas had 2 ppm manganese in hair. Caucasians living in the same manganese-rich area had 2.5 ppm manganese in hair, compared to 0.5 ppm manganese in non-manganese areas. Measurements of manganese in hair and blood of Groote Eylandt Aborigines showed that the population had a high exposure to manganese, but did not distinguish between those individuals affected/unaffected by the neurological condition, Groote Eylandt Syndrome.

## INTRODUCTION

Aborigines living at Angurugu, a small township on Groote Eylandt (GE), in the Gulf of Carpentaria, Australia, have a high incidence of neurological disturbances referred to as the Groote Eylandt Syndrome (Cawte, 1984). It has been suggested that GE Syndrome may be caused by environmental manganese, as similar neurological symptoms have been found in South American miners suffering from manganese poisoning (Cotzias et al., 1968). The Angurugu population is exposed to an environment naturally high in manganese. Soil samples collected from the township contain up to 4% manganese as $MnO_2$ in the mineral pyrolusite (Florence et al., 1987). The Groote Eylandt Mining Company (GEMCO) has mined manganese since the early 1960s.

Manganese, absorbed through the lungs and gastrointestinal tract is cleared

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quickly from the blood and deposited in tissue, particularly the liver, kidney, pancreas and brain. The neurotoxicity of manganese may result from its ability to catalyse the oxidation of dopamine, causing depletion of this neurotransmitter (Donaldson, 1987; Florence and Stauber, 1989). Manganese is excreted in the faeces via bile, with very little being excreted in the urine (Piscator, 1979; Keen et al., 1984). Because manganese has a short biological half-life (4 days for the fast fraction (30%) and 40 days for the slow fraction), monitoring manganese at the low levels found in blood and urine can only indicate relatively recent exposure.

The most convenient record of manganese exposure over a few months is the manganese concentration in scalp hair, which grows at a rate of 1–2 cm per month (Hopps, 1977). Problems in using scalp hair to monitor exposure include pre-treatment of the hair before manganese analysis, and the need to distinguish between endogenous (manganese that enters the hair via the blood supply to the hair follicle) and exogenous sources of manganese, including dust and sweat (Chittleborough, 1980).

To determine whether the Aboriginal population of Angurugu had a significantly higher manganese intake than other populations, we monitored manganese in scalp hair of over 100 inhabitants (Stauber et al., 1987). We found that Aborigines living at Angurugu had much higher hair manganese than those from non-manganese areas, e.g., Gove Peninsula. Although Caucasians at Angurugu had lower hair manganese than Aborigines, it was much higher than hair manganese for Caucasians living in Sydney. To interpret these scalp-hair manganese results, it was necessary to determine the contribution of exogenous manganese to the total manganese measured in hair. In this paper we examine sources of exogenous manganese in hair, including the solubility of manganese from Groote Eylandt roadside dust in sweat and its incorporation into Aboriginal and Caucasian hair. A number of methods to overcome the problem of exogenous manganese in hair were also examined.

## EXPERIMENTAL

### *Hair collection and analysis*

Scalp hair (collected from the nape of the neck) and pubic hair were cut and washed in 25 ml of non-ionic detergent (0.5% Triton X-100) in an ultrasonic bath for 15 min. Hair samples were rinsed with distilled water through a pierced Whatman 542 filter paper, rinsed with methanol and air-dried before manganese determination by neutron activation analysis (NAA). Dust washed from the hair was collected and analysed for manganese by inductively coupled plasma emission spectroscopy (ICP). To ensure that all the dust was removed by the washing procedure, GE roadside dust was rubbed into the hair, shaken overnight and the hair washed in the same manner. Analysis showed that the washing procedure effectively removed all surface dust, and gave reproducible results for multiple hair samples from the same person.

*Endogenous and exogenous manganese in hair*

Manganese in dust trapped in the hair before sampling, may be solubilized in sweat, incorporated into the hair shaft and measured as endogenous manganese. To determine the extent to which this exogenous source of manganese contributes to total manganese in hair, experiments using both Aboriginal and Caucasian hair were conducted.

*Solubility of manganese from dust in sweat*

Roadside dirt from Angurugu was collected and sifted through a 100 mesh sieve. It contained 4.4% Mn. Sweat from two sources was used in the following experiments:

(i) Synthetic sweat (pH 5.2). The composition of synthetic sweat is shown in Table 1 (Stauber and Florence, 1987). Synthetic sweat was used with/without the addition of 0.4 mg $ml^{-1}$ protein mixture (containing 0.05 g albumin $ml^{-1}$ and 0.03 g globulin $ml^{-1}$) and sebum (0.025 ml $ml^{-1}$ sweat). The sebum stock solution contained 3% palmitic acid, 5% palmitic acid methyl ester, 2% linoleic acid, 0.1% cholesterol and 0.2% cholesterol hexanoate, dissolved in 10 ml of warm ethanol.

(ii) Sauna sweat. In a sauna, forearm sweat was collected into acid-washed containers (Stauber and Florence, 1988).

The solubility of manganese from dust in sweat was determined by shaking a large excess of Angurugu roadside dust in sweat for 1–6 days. The solution was filtered through an acid-washed 0.45 $\mu$m membrane filter and manganese in the filtrate determined by ICP.

As most of the manganese in Angurugu dust was present as $MnO_2$, the ability of sweat to dissolve manganese from a standard suspension of freshly prepared $MnO_2$ (Stauber and Florence, 1985) was measured using Milli-Q water, sauna sweat, synthetic sweat, and synthetic sweat without lactic acid. Sweat was shaken overnight with 0.1 ml of 0.1 *M* $MnO_2$ suspension, followed by centrifugation for 20 min in an Amicon CF-25 Centriflo membrane ultrafilter cone, which retains molecules greater than 25000 MW. Manganese (II) in the ultrafiltrate was determined by ICP. To identify which component of sweat was reducing Mn(IV) to Mn(II), the experiment was repeated using solutions of lactic acid, NaCl, urea, histidine·HCl and citrulline in Milli-Q water (pH 5.2) at concentrations found in synthetic sweat.

*Lipid solubility of manganese in sweat*

Ten millilitres of synthetic sweat ( $\pm$ 0.4 mg protein mixture $ml^{-1}$) containing 5 $\mu$g Mn(II) $l^{-1}$ was shaken for 5 min in 10 ml of n-octanol. After standing for 15 min, the aqueous phase was separated and manganese in the aqueous phase determined by ICP.

TABLE 1

Composition of synthetic sweat*

| Compound | Concentration (mg $l^{-1}$) |
|---|---|
| NaCl | 800 |
| KCl | 912 |
| $CaSO_4 \cdot 2H_2O$ | 200 |
| $MgSO_4 \cdot 7H_2O$ | 88 |
| $NaH_2PO_4 \cdot 2H_2O$ | 31 |
| $FeSO_4 \cdot 7H_2O$ | 11 |
| Lactic acid | 1472 |
| Urea | 300 |
| Histidine·HCl | 240 |
| Alanine | 920 |
| Glycine | 280 |
| Citrulline | 152 |
| Urocanic acid | 76 |

*Synthetic sweat was adjusted to pH 5.2 with 0.2 *M* $Na_2CO_3$.

*Incorporation of manganese from sweat into hair*

To determine how much manganese could be incorporated into hair from exogenous sources, Aboriginal and Caucasian hair was shaken for up to one month in sweat containing radiolabelled manganese ($^{54}Mn$(II) or $^{54}MnO_2$). The total manganese concentration in sweat ranged from < 1 to 5000 $\mu$g Mn $l^{-1}$. After shaking, the hair was washed as usual in Triton X-100, dried and acid digested in 2 ml 15 *M* $HNO_3$ and 0.5 ml 72% $HClO_4$ (Supaprur) to strong fumes of $HClO_4$. Exogenous manganese incorporated into the hair was determined by counting in a gamma well counter, and compared to total manganese in hair, measured by ICP.

To establish whether more manganese is incorporated into hair when sweat evaporates, hair was shaken overnight in synthetic sweat containing $^{54}Mn$ (500 $\mu$g Mn(II) $l^{-1}$) and air-dried during the day to simulate evaporation. The procedure was repeated for 5 days, before final washing of the hair, acid digestion and manganese determination by gamma counting. In another experiment, hair was shaken for 3 days in $^{54}Mn$(II)-sweat solution, and allowed to stand for 4 days, before measurement of manganese uptake.

*Incorporation of manganese from dust via sweat into hair*

Hair was shaken for 6 days with 10–500 mg of Angurugu roadside dust in 25 ml of sauna sweat. Manganese uptake into hair was measured by ICP, after washing in Triton X-100 and acid digestion in $HNO_3/HClO_4$.

*Manganese incorporation along the length of hair*

To examine whether manganese incorporation from sweat was constant along the length of the hair, root-to-tip hair samples were collected and treated as follows:

(1) Hair was shaken with synthetic sweat containing 500 $\mu$g Mn(II) $l^{-1}$ (with $^{54}$Mn).

(2) Hair was shaken for 6 days in synthetic sweat and 500 $\mu$g Mn(II) $l^{-1}$ (with $^{54}$Mn), air-dried and the roots dipped in a beaker of synthetic sweat for 6 days.

(3) The roots of the hair were placed in a beaker of synthetic sweat and 500 $\mu$g Mn(II) $l^{-1}$ (with $^{54}$Mn) for 6 days. After treatment, the hair was cut into 1.5 cm lengths, washed with Triton X-100 and acid digested; the exogenous manganese along the length of the hair was then determined by gamma counting. Manganese in untreated hair, cut into 1.5 cm lengths, was also determined by ICP.

*Selective leaching of exogenous manganese from hair*

Experiments to selectively leach exogenous manganese incorporated into hair from dust and sweat were carried out. Hair, which had been shaken with radioactive $^{54}$Mn (500 $\mu$g Mn(II) $l^{-1}$) in sweat for 1–6 days, was washed as usual with Triton X-100. The hair was then divided into aliquots and washed with 0.1 *M* solutions of HCl, cysteine-HCl, CyDTA, EDTA, oxalic acid, ascorbic acid, penicillamine, diethyldithiocarbamate, hydroquinone and $H_2O_2$, with ultrasonication for up to 6 h. Other leaching agents included 0.05 *M* dithiodipyridine, 0.02 *M* indomethacin, 0.01 *M* potassium ethylxanthogenate, 0.01 *M* oxine, 50% ethanol and 1% sodium lauryl sulphate. To measure the effect of leaching agents on endogenous manganese, unexposed hair was washed with Triton X-100, followed by various leaching agents and the remaining manganese in hair measured by ICP.

*Manganese in blood*

Manganese in whole blood from Aborigines and Caucasians on Groote Eylandt was determined by atomic absorption spectroscopy (AAS) at the Prince of Wales Hospital, Sydney, New South Wales, Australia. Iron in blood was determined by NAA. Haemoglobin and ferritin in blood were determined by the Mansfield Pathology Laboratories, Sydney.

## RESULTS

*Solubility of manganese from dust in sweat*

Concentrations of manganese in different sweat solutions after shaking 50 mg Angurugu roadside dust for 1 day in 25 ml of sweat are shown in Table 2.

TABLE 2

Solubility of manganese from Groote Eylandt roadside dust in sweat*

| Solution | Manganese ($\mu g\ l^{-1}$) |
|---|---|
| Milli-Q water | 80 |
| Synthetic sweat | 800 |
| Synthetic sweat - protein | 640 |
| Synthetic sweat - sebum | 840 |
| Sauna sweat | 1120 |

*50 mg dust - 25 ml sweat, shaken for 1 day. Dust contained 4.4% Mn.

Longer shaking times (up to 6 days) and different amounts of dust (10–500 mg) did not increase the manganese concentrations in sweat. Synthetic sweat dissolved ten times more manganese from dust than Milli-Q water, under the same conditions. The addition of protein and sebum to synthetic sweat had little effect. Slightly more manganese dissolved in sauna sweat.

These results suggest that sweat contains reducing agents that are able to dissolve the Mn(IV) and Mn(III) present in the pyrolusite ore in the dust. The ability of sweat to dissolve manganese from a standard suspension of $MnO_2$ was measured using synthetic sweat, sauna sweat and Milli-Q water (Table 3). Milli-Q water did not substantially dissolve the manganese, whereas in the sweat solutions there was significant and equal dissolution. Lactic acid, histidine-HCl, citrulline and urocanic acid were the major components of sweat which reduced $MnO_2$.

TABLE 3

Solubility of $MnO_2$ in sweat and sweat components*

| Solution[b] | Manganese ($\mu g\ ml^{-1}$) |
|---|---|
| Milli-Q water | 0.01 |
| Sauna sweat | 98 |
| Synthetic sweat | 102 |
| Synthetic sweat (without lactic acid) | 116 |
| Lactic acid | 109 |
| Histidine hydrochloride | 120 |
| Citrulline | 42 |
| Urocanic acid | 50 |
| Sodium chloride | 0.26 |
| Urea | 0.07 |

*0.1 ml of 0.1 *M* $MnO_2$ shaken with 1 ml solution for one day.

[b]Concentration of lactic acid, urea, etc. were the same as that present in synthetic sweat (Table 1). pH was adjusted to 5.2 as for synthetic sweat.

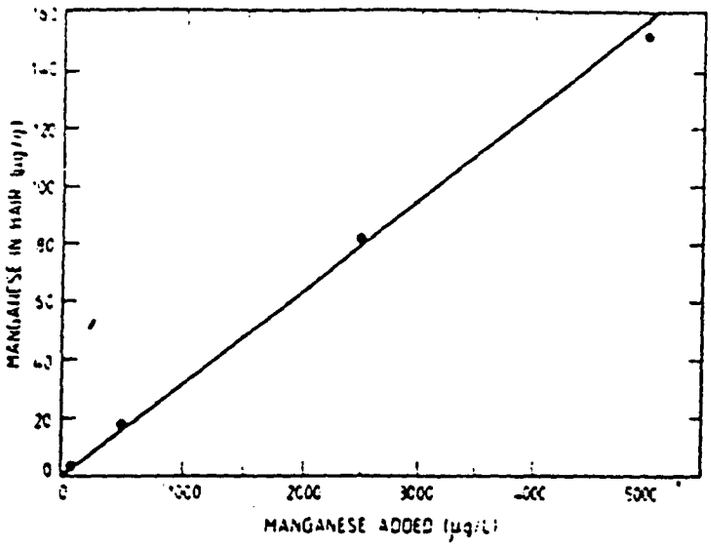


Fig. 1. Uptake of manganese from synthetic sweat into hair.

*Lipid solubility of manganese in sweat*

Solubility in n-octanol is often used to simulate lipid solubility. However, when a solution of manganese in synthetic sweat was shaken with n-octanol, manganese was not extracted, indicating that manganese in sweat was not in a lipid-soluble form.

*Incorporation of manganese from sweat into hair*

When hair was shaken for 10 days with synthetic sweat containing 0–5000 µg Mn(II) $l^{-1}$, the uptake of manganese into hair was proportional to manganese concentration (Fig. 1). When 300 mg of hair was shaken in 25 ml synthetic sweat containing 500 µg Mn $l^{-1}$ (as Mn(II) or $MnO_2$), about 40% of the manganese was incorporated into hair (~ 17 µg Mn $g^{-1}$ Triton X-100 washed hair). Aboriginal and Caucasian hair incorporated similar concentrations of manganese. There

TABLE 4

Incorporation of manganese into hair from Groote Eylandt roadside dust, shaken in sauna sweat*

| Weight of dust (mg) | Manganese incorporated into hair (ppm) |
|---|---|
| 10 | 54 |
| 50 | 175 |
| 150 | 200 |
| 500 | 380 |

*10–500 mg dust (4.4% Mn) and 250 mg hair shaken for 6 days in 25 ml sauna sweat.

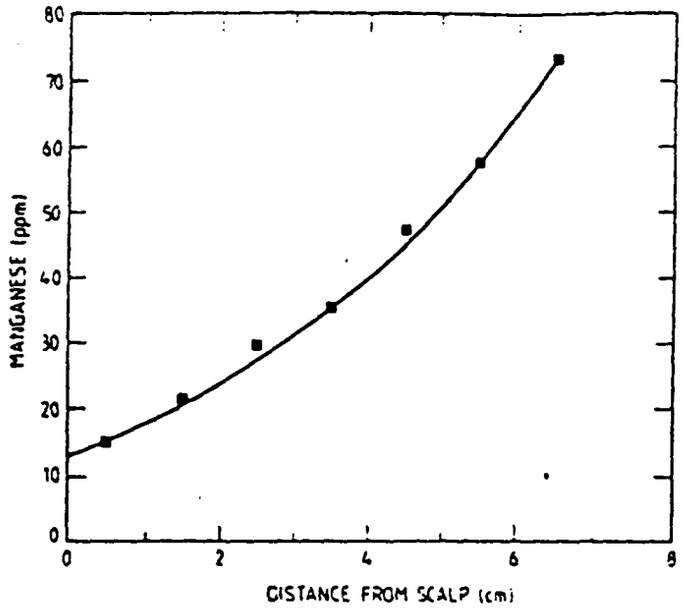


Fig. 2. Manganese along the length of hair from one Aborigine affected by Groote Eylandt Syndrome.

was no increase in uptake when the manganese solution was replaced each day for 10 days. Washing the hair with Triton X-100 after each daily exposure to sweat and manganese (to simulate daily hair shampooing) did not affect uptake of manganese.

Uptake of manganese into hair increased with increased shaking time for 4–5 days, after which additional shaking (up to 1 month) led to only a small increase. Triton X-100 was no more effective than Milli-Q water in removing exogenous manganese from hair after long shaking periods ( > 10 days).

Hair shaken with Mn(II) in sweat for 3 days, rinsed with Milli-Q water, allowed to stand for 4 days, then subjected to the standard washing procedure, did not incorporate more manganese than hair shaken for 3 days and measured immediately. Moreover, manganese was just as leachable with Triton X-100 after the 7 day experiment, suggesting that manganese does not diffuse slowly into the hair cortex.

Only slightly more manganese was incorporated into hair (13 $\mu$g Mn $g^{-1}$ hair) when Mn(II)-containing sweat was allowed to evaporate on the hair each day for 5 days, compared to hair shaken for 5 days without drying (10 $\mu$g Mn $g^{-1}$ hair).

When Angurugu roadside dust, sauna sweat and hair were shaken together for 6 days, incorporation of manganese into hair increased with increasing amounts of dust. This increase was not linear (Table 4). Up to 380 $\mu$g Mn $g^{-1}$ hair was incorporated into hair in the presence of 500 mg dust and 250 mg hair. It is unlikely that such large amounts of dust would be associated with Aboriginal hair. Dust collected during the washing of Aboriginal hair with Triton X-100, contributed only ~ 14 $\mu$g Mn $g^{-1}$ hair.

*Manganese incorporation along the length of hair*

Changes in manganese concentration along the length of hair were measured in a number of Aborigines and Caucasians from Groote Eylandt. Manganese always increased along the length of hair from roots to tips, reaching 80 ppm in the distal ends of some Aboriginal hair. Figure 2 shows a typical plot of manganese along the length of hair from one Aborigine affected by GE Syndrome. This increase in manganese from roots to tips may be due to:

(a) age-related damage to the outer cuticle layer of the hair, which enables more exogenous manganese to be absorbed towards hair tips;

(b) elution of sweat from the scalp, which travels along the hair, dissolves manganese in dust particles on the hair and concentrates manganese towards the tips, i.e. hair acts like a chromatographic column (wick) with sweat as the eluant.

The results of experiments to test the wick effect are presented in Table 5. When the roots only of hair were placed in a beaker of synthetic sweat containing 500 $\mu$g Mn(II) $l^{-1}$ with the distal ends hanging over the side of the beaker, it had less manganese along its length towards the tips (treatment 3, Table 5). Manganese increased along the length of hair towards the tips for hair which had previously been shaken with manganese in sweat and the roots placed in a beaker of synthetic sweat (no manganese) (treatment 2, Table 5). However, manganese also increased along the length of hair from roots to tips,

TABLE 5

Incorporation of manganese along the length of hair

| Hair section[a] | Manganese ($\mu$g g hair) | | | |
|---|---|---|---|---|
| | Untreated | Treatment 1[b] | Treatment 2[c] | Treatment 3[d] |
| Roots | 0.1 | 4.3 | 2.6 | 41.8 |
| | 0.2 | 9.8 | 6.8 | 37.0 |
| | 0.6 | 10.7 | 8.2 | 30.1 |
| | 0.8 | 10.9 | 8.8 | 27.3 |
| | 0.7 | 11.3 | 8.6 | 21.7 |
| | 1.2 | 12.0 | 12.1 | 20.6 |
| | 1.2 | 13.3 | 12.8 | 20.6 |
| | 1.2 | 11.6 | 14.9 | 19.4 |
| Ends | 1.2 | 15.6 | 16.4 | 22.7 |

[a] Each hair section was 1.5 cm in length.

[b] 730 mg hair was shaken for 6 days in 25 ml synthetic sweat containing 500 $\mu$g Mn(II) $l^{-1}$ (with $^{54}$Mn).

[c] 740 mg hair was shaken for 6 days in 25 ml synthetic sweat containing 500 $\mu$g Mn(II) $l^{-1}$ (with $^{54}$Mn) then roots dipped in a beaker of synthetic sweat for 6 days.

[d] The roots of 737 mg hair were dipped in a beaker of synthetic sweat containing 500 $\mu$g Mn(II) $l^{-1}$ (with $^{54}$Mn) for 6 days.

when hair was simply shaken in a manganese–sweat solution, and analysed immediately (treatment 1, Table 5). This suggests that increasing manganese towards hair tips is not due to a wick effect, but more likely due to increasing damage to the hair cuticle with age (i.e., distance from the scalp) allowing more exogenous manganese to be absorbed in the cortical cells.

*Selective leaching of exogenous manganese*

The effectiveness of a number of chemical chelators, thiols, acids and solvents in removing exogenous manganese from hair are shown in Table 6. The most effective leaching agents were 0.1 *M* HCl and 0.1 *M* cysteine-HCl, which removed 71% and 51% of the exogenous manganese, respectively, but also removed much of the endogenous manganese. Ultrasonication for 1 h with 0.1 *M* CyDTA did not remove any endogenous Mn, but only extracted 15–40% of the exogenous manganese, with the percentage extracted decreasing with increasing exposure to manganese. The longer the hair was shaken with manganese in sweat, the less effectively the agents removed manganese. The

TABLE 6

Percentage manganese removed from hair by various leaching agents[a]

| Leaching agent | Percentage exogenous manganese removed |
|---|---|
| HCl | 71 |
| Cysteine-HCl (pH 1.3) | 51 |
| Cysteine-HCl (pH 1.3) - 50% ethanol | 42 |
| CyDTA[b] (pH 5.5) | 37 |
| HCl (0.025 *M*, pH 1.3) | 34 |
| EDTA | 33 |
| Dithiodipyridine (0.05 *M*) | 33 |
| Hydroquinone | 21 |
| Diethyldithiocarbamate | 21 |
| Oxalic acid | 21 |
| Sodium lauryl sulphate (1%) | 20 |
| $H_2O_2$ - CyDTA | 19 |
| $H_2O_2$ | 16 |
| Indomethacin | 16 |
| Oxine (0.01 *M*) | 12 |
| Ascorbic acid | 10 |
| Potassium ethylxanthogenate | 4 |
| Ethanol (50%) | 3 |
| Penicillamine | 3 |
| Cysteine HCl (pH 5.1) | 2 |

[a] Hair was shaken for 1–3 days in synthetic sweat containing 500 μg Mn $l^{-1}$ (labelled with $^{54}$Mn). All leaching agents used were 0.1 *M*, unless otherwise stated.
[b] Cyclohexylenedinitrilotetraacetic acid.

anionic detergent, sodium lauryl sulphate (SDS) only removed 20% of exogenous manganese after 1 h ultrasonication at 25°C and 58% at 80°C. Potassium hydroxide (3 *M*), which extracts the keratins (protein components of hair), removed only 60% of exogenous manganese after hair was shaken for 7 days.

## DISCUSSION

Large amounts of manganese may be dissolved by sweat from Angurugu roadside dust and incorporated into hair. This source of exogenous manganese in hair may partly account for the elevated manganese levels found in the hair of Aborigines and Caucasians living in Angurugu (Stauber et al., 1987). The average concentration of manganese in hair dust collected from Angurugu was 1.7%. This could lead to 500 $\mu$g Mn $l^{-1}$ dissolved in sweat (Table 2) and, from Fig. 1, an incorporation of up to 15 $\mu$g Mn $g^{-1}$ hair. Larger amounts of dust in hair, the presence of roadside dust (which is 4% manganese) and evaporation of sweat on hair could all lead to an even greater incorporation of manganese in hair via this exogenous route.

To obtain reliable estimates of manganese taken up from blood in the hair follicle, manganese in hair from exogenous sources must be eliminated. If manganese from exogenous sources is bound only to the outer cuticle layer of hair, and does not penetrate the hair cortex, or is bound to different functional groups on amino acids than endogenous manganese, it may be possible to remove exogenous manganese from hair before analysis. Bos et al. (1985) and Li and Malmqvist (1985), using microbeam particle induced X-ray emission (PIXE), studied the distribution of iron, lead, sulphur, zinc and copper across single hairs. They found that some elements, including zinc, sulphur and copper, have a homogeneous distribution across the hair, whereas endogenous iron and lead have higher concentrations at the hair periphery.

Although the distribution of manganese across the hair has not been determined, there is some evidence from acid staining wool, that manganese binds preferentially to orthocortical cells, perhaps to the amino acid tyrosine (Corbett and Yu, 1964). If this is the case, selective leaching of exogenous manganese from hair will be very difficult. We were unable to find a leaching agent that could remove exogenous manganese, without affecting endogenous manganese. Buckley and Dreosti (1984) were unable to find a washing procedure that removed only exogenous zinc from hair. They concluded that the location and binding of endogenous and exogenous zinc in hair may not be significantly dissimilar. Nishiyama and Nordberg (1972) also found that acid and alkaline washes could not distinguish endogenous and exogenous cadmium in hair. Moreover, endogenous cadmium was more easily eluted than cadmium adsorbed to hair from exogenous sources.

Other ways of overcoming the problem of exogenous manganese include sampling the 1–2 cm of hair closest to the scalp or measuring manganese along the length of hair and extrapolating back to zero length at the scalp. The latter

TABLE 7

Manganese in scalp and pubic hair

| Subjects | Mean Mn (ppm) | |
|---|---|---|
| | Scalp[a] | Pubic |
| Caucasians - Sydney | 0.5 ± 0.2[b] | 1.3 ± 0.5 |
| GEMCO workers | 2.2 ± 0.8 | 3.0 ± 0.4 |
| Caucasians - Angurugu | 2.5 ± 0.7 | 7.9 ± 4.1 |
| Affected Aborigines | 9 ± 5 | 25 ± 13 |
| Unaffected Aborigines | 16 ± 12 | 23 ± 6 |

[a]Extrapolated to zero length.
[b]One standard deviation.

method enables an estimate of the manganese concentration in hair as it emerges from the scalp, before manganese from exogenous sources interferes. We used this method to reassess manganese in scalp hair from Groote Eylandters. Scalp-hair manganese, extrapolated back to zero, and pubic-hair manganese in Angurugu Aborigines affected/unaffected by GE Syndrome, Caucasians living at Angurugu and some Caucasian GEMCO workers are shown in Table 7. Manganese in both scalp hair and pubic hair of Angurugu Aborigines was about 20 times the non-exposed values of Sydney Caucasians. There was no significant difference in hair manganese between Aborigines with GE Syndrome and those without the disease. Scalp-hair manganese for both the Caucasians at Angurugu and the GEMCO workers were three–six times the Sydney values. These results suggest that the Angurugu population, particularly the Aborigines, have a high intake of manganese.

Higher hair manganese in Aborigines than Caucasians may be due to their different lifestyles, e.g., Aborigines "close-to-the-earth" living or due to differences in their diets. We measured manganese in some traditional food samples, including yams and fruit from the old Angurugu garden areas (Florence et al., 1987). Some of the foods were exceptionally high in manganese, e.g., one 20 g yam would supply 13 mg of manganese; three times the recommended daily allowance of this element. One litre of boiled billy tea, which is part of the Aborigines' staple diet, contained 6–7 mg manganese, five times more than brewed tea drunk by Caucasians.

To determine whether manganese in scalp hair gives a reliable estimate of manganese intake, manganese in blood from the same groups was determined (Table 8). The Caucasians living at Angurugu and the GEMCO workers had blood manganese values within the normal range for Sydney controls (6–12 $\mu$g Mn $l^{-1}$). Angurugu Aboriginals had elevated levels of manganese in blood, although there was no significant difference between those affected and unaffected by GE Syndrome. The affected Aboriginals also had low haemoglobin (normal range 12–18 g $dl^{-1}$) and low ferritin (normal range 25–150 (F) and 75–260 (M) $\mu$g $l^{-1}$), indicative of low iron status (anaemia). Anaemia,

TABLE 8

Mean manganese, iron, haemoglobin and ferritin in blood of Groote Eylandt inhabitants

| Subjects | Mn ($\mu g\ l^{-1}$) | Fe ($\mu g\ l^{-1}$) | Hb ($g\ dl^{-1}$) | Ferritin ($\mu g\ l^{-1}$) |
|---|---|---|---|---|
| GEMCO workers | 8.4 ± 2.3 | 431 ± 46 | 15.7 ± 0.9[a]<br>14.0 ± 0.6[b] | 146 ± 68 |
| Caucasians, Angurugu | 7.3 ± 2.3 | 388 ± 32 | 15.1[a]<br>12.9 ± 0.9[b] | 69 ± 40 |
| Affected Aborigines | 36.1 ± 10.2 | 294 ± 73 | 8.9[a]<br>11.0 ± 0.6[b] | 11 ± 11 |
| Unaffected Aborigines | 18.9 ± 6.5 | 403 ± 69 | 15.7 ± 1.0[a]<br>12.3[b] | 161 ± 161 |

[a]Males.
[b]Females.

chronic infections, high alcohol intake and low dietary calcium and zinc all increase the toxic effects of manganese (Schager et al., 1974; World Health Organization 1981).

Measurement of manganese in blood and hair probably only indicates manganese in the body that is in readily exchangeable pools, such as soft tissue. Because hair and blood determinations can only indicate relatively recent manganese exposure, we were unable to distinguish between those individuals affected/unaffected by GE Syndrome. The exposure which caused the neurological symptoms may have occurred many years previously, perhaps in early childhood. A sample of umbilical cord blood taken from a young Angurugu mother had 40 $\mu g\ Mn\ l^{-1}$. Determination of manganese in scalp hair does show, however, that the Angurugu population has a high intake of manganese. Only 2% of the population are susceptible; perhaps only those individuals with a genetic difference in manganese metabolism which leads to enhanced manganese uptake or reduced manganese clearance from the body.

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# ATTACHMENT C-5

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# An Analysis for Blood Manganese Used to Assess Environmental Exposure

**G. A. Hams and J. K. Fabri**

In this graphite-furnace atomic-absorption spectrometric method for measuring manganese in whole blood, we use a pyrolytic platform to minimize interference by sample matrix. For optimal sample ashing we denature the sample within the furnace with nitric acid and use oxygen as the purge gas at low temperatures. The mean manganese concentration found in blood from 15 unexposed city dwellers was 215 (2 SD 135) nmol/L. By comparison, the range of manganese concentrations in blood sampled from a group of Australian aborigines living near a surface manganese ore deposit on Groote Eylandt, Northern Territory, was much higher (median 405 nmol/L, range 175 to 990 nmol/L).

**Additional Keyphrases:** *graphite-furnace atomic-absorption spectrometry · reference values · environmental hazards · trace elements · toxicology · screening*

Manganese is an essential trace element required for the maintenance of biological function in humans and other animals. Manganese neurotoxicity has been recognized after cases of exposure to dust derived from manganese ores. The symptoms of the neurotoxicity, which resemble Parkinson's disease, persist even when exposure is minimized and the body burden of manganese decreased. The nature of the neurological insult has not been determined, but possibly it is linked to the presence of neurotoxic dopamine oxidation products and hydrogen peroxide (*1*). A comprehensive review of manganese metabolism as related to occupational and environmental exposure has been published (*2*).

Several graphite-furnace atomic-absorption spectrometric (GFAAS) methods for manganese have been reported (*3*). Direct methods for blood samples are characterized by large nonatomic absorbances during atomization (*4*) or by accumulation of residue within the atomizer (*5*). One can correct for large nonatomic absorbance signals by using a Zeeman instrument, but this procedure fails to compensate for loss of manganese compounds that may be present in the gas phase during the atomization ramp. For GFAAS, nonatomic signals should be eliminated if possible, or at least minimized. Poor analysis precision owing to sample residue remaining after atomization must be addressed by modifying sample preparation and ashing. Wet digestion with various acids before sampling for furnace atomization is theoretically an answer, but this approach has associated contamination risks.

Hoenig (*6*) recently reported the use of a combination of low-temperature oxygen ashing, nitric acid matrix modification, and furnace platform technique for the analysis for selenium in whole blood. Here we describe a modification of that technique for use in analysis for manganese.

## Materials and Methods

### Instrumentation

For the analysis we used a Model AA975 spectrometer equipped with a Model GTA95 graphite furnace with autosampler, all from Varian P/L., Mulgrave, Victoria, Australia. The solid pyrolytic graphite platforms were installed in pyrolytically coated graphite-furnace tubes.

In developing the analysis we used a remote computer running the Varian Tape Graphics program for the AA975. This enabled video display of both atomic and nonatomic (deuterium) signals during atomization. For routine analyses, atomic absorption signals corrected for background were displayed on the video screen of the GTA95.

Gases supplied to the furnace were medical-grade oxygen and "ultra high purity" argon (Commonwealth Industrial Gases, Surry Hills, N.S.W., Australia).

We stored polypropylene sampler cups (Varian no. 99-100282-00) in nitric acid before washing in metal-free water, drying, and using them as containers for the diluted blood samples.

### Chemicals

A working manganese standard in dilute nitric acid (73.0 nmol of manganese per liter, in 7 g·kg $HNO_3$) was prepared by serially diluting a manganese atomic absorption standard solution (Sigma Chemical Co., St. Louis, MO).

Analytical-grade concentrated nitric acid (700 g·kg) was used for matrix modification and preparation of standards.

Trace Metal Laboratory, Department of Clinical Chemistry, The Prince of Wales Hospital, High Street, Randwick, N.S.W. 2031, Australia.

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Water for analytical use (redistilled from a town water distillate and then de-ionized just before use) contained less than 2 nmol of manganese per liter. Sample diluent was prepared by adding one drop of Triton X-100 surfactant (BDH Chemicals Australia Pty. Ltd., Port Fairy, Victoria, Australia) to 40 mL of distilled de-ionized water. We checked the sample diluent for contamination before diluting any samples and used it only if no manganese absorption peaks were seen during atomization.

Polystyrene tubes containing lithium heparin (Mallinckrodt Pty. Ltd., Gladesville, N.S.W., Australia) were used to store the blood samples. These tubes did not add detectable manganese to normal whole blood.

## Blood Collection

We collected 15 control blood samples from laboratory workers and 40 blood samples from the exposed population on Groote Eylandt, Northern Territory, Australia. Venous blood samples were collected with plastic syringes (Terumo Australia, Pty. Ltd., Melbourne) fitted with 26-gauge stainless-steel hypodermic needles. The syringe-needle assembly was rinsed with the patient's blood before we collected the sample to be used for manganese analysis.

Heparinized samples were stored at 4 °C if the analysis was to be done within seven days; otherwise, they were frozen. Freezing and thawing blood samples several times did not affect the measured manganese, but it occasionally led to the formation of small clots, which caused sample-handling difficulties.

We diluted 100 μL of blood with 700 μL of sample diluent, using an air-displacement pipette (Finnpipette, Lab Supply, Marrickville, N.S.W., Australia) and performed the analysis within 60 min.

## Analysis

The spectrometer was operated at 279.5 nm with a slit width of 0.2 nm. The lamp current was 5 mA. Table 1 lists the settings for the graphite furnace controller. The automatic pipette was programmed to deliver a total volume of 19 μL for each analysis (see Table 2).

The analysis was routinely calibrated with aqueous standards (range 0 to 700 nmol of manganese per liter of whole blood) after equivalence had been established between aqueous standard and dilute-blood-sample sensitivities. Occasionally, as the graphite furnace and platform aged, equivalence could not be shown. On these occasions the analysis was calibrated by the method of standard additions.

Diluted samples and standards were analyzed in duplicate, and the mean absorbance was used for calculation. Repeat analyses were made when the individual atomization readings deviated from the mean by >10%.

Table 1. Settings for Furnace Operation

| Step no. | Temp, °C | Time between steps, s | Gas flow, L/min | Gas |
|---|---|---|---|---|
| 1 | 150 | 5.0 | 3.0 | Oxygen |
| 2 | 200 | 15 | 3.0 | Oxygen |
| 3 | 250 | 30 | 3.0 | Oxygen |
| 4 | 300 | 20 | 3.0 | Oxygen |
| 5 | 300 | 2.0 | 3.0 | Argon |
| 6 | 400 | 10 | 3.0 | Argon |
| 7 | 800 | 10 | 3.0 | Argon |
| 8 | 800 | 15 | 3.0 | Argon |
| 9 | 800 | 2.0 | 0 | Argon |
| 10 | 2600 | 1.0 | 0 | Argon[a] |
| 11 | 2600 | 1.0 | 0 | Argon[a] |
| 12 | 2600 | 8 | 0 | Argon[a] |
| 13 | 40 | 5.1 | 3.0 | Argon |
| 14 | 40 | 5.0 | 3.0 | Argon |

[a] Absorbance readings were taken over steps 10–12, inclusive. The injection temp was 150 °C

Table 2. Settings for Automatic Pipettor Operation

| Solution | Sample or std[a] vol | Blank vol[b] | Modifier vol[c] |
|---|---|---|---|
| | | μL | |
| Blank | — | 1 | 18 |
| Std 1 | 4 | 1 | 14 |
| Std 2 | 8 | 1 | 10 |
| Std 3 | 12 | 1 | 6 |
| Sample | 10 | 1 | 8 |

[a] Standard manganese solution 73 nmol/L, see text.
[b] Blank solution; nitric acid 700 g/kg.
[c] Modifier solution; de-ionized water containing a trace of Triton X-100.

## Results

Figure 1, which shows the atomic (hollow cathode minus deuterium) and the nonatomic deuterium signals during the atomization of a dilute blood sample, illustrates the low background intensity and the good separation of the atomization signals.

The coefficient of variation was 11.3% for analysis of a blood sample having a manganese concentration of 160 nmol/L. This precision estimate was based on 16 analytical runs on different days, with use of freshly prepared standards.

The atomic absorbance signal produced by this sample relative to the sample diluent was 0.04 peak-height unit. The absolute detection limit of the analysis (based on two standard deviations of the baseline noise) was 20 nmol/L. We considered the working detection limit to be 40 nmol/L.

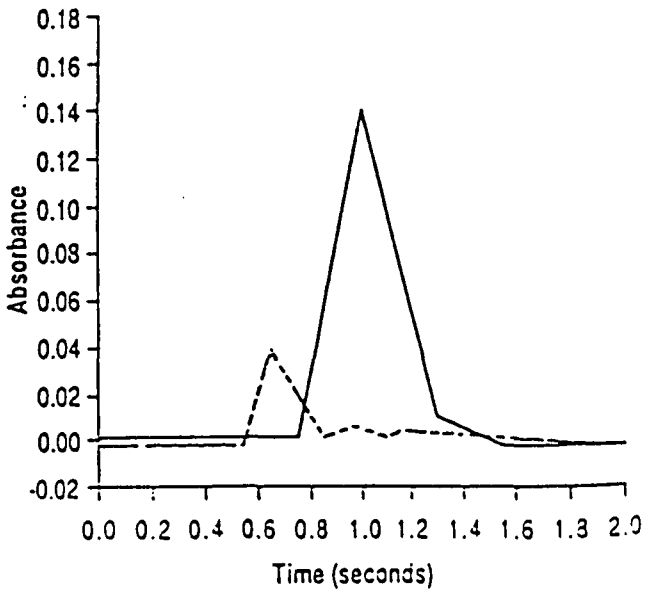


Fig. 1. Stylized atomization signals from a diluted blood sample
*Solid line*, the atomic absorption signal. *Broken line*, the background (deuterium signal)

The mean value for the laboratory workers was 215 (2 SD 135) nmol/L. The mean blood manganese concentration of the samples collected from the exposed population on Groote Eylandt was 490 nmol/L, and there was considerable asymmetry in the distribution of these data (range 175–990 nmol/L, median 405 nmol/L).

## Discussion

This analysis has advantages over previously described techniques. The samples were analyzed after simply diluting them with water containing a trace of Triton X-100. Use of other reagents was minimized to minimize contamination risks. Low-temperature ashing in oxygen, combined with nitric acid matrix modification and use of the platform technique, resulted in an analysis characterized by low background signals. A particular advantage of the platform technique was the equivalence in analysis sensitivity observed for aqueous and dilute blood samples.

We find that routine analytical runs leave no significant residue on the platform if diluted blood is atomized alternately with sample diluent or aqueous standard. This approach allows continuous monitoring of baseline stability and analysis sensitivity in addition to assisting in cleaning the platform.

Reference intervals for blood manganese have been published (*4*, *7*, *8*) and are of the same order as found for this study's controls.

The increased blood manganese found in the Groote Eylandt aborigines is interesting. Groote Eylandt is characterized by the presence of a manganese ore body, which outcrops in the area surrounding the aboriginal settlement. Neutron activation analysis of soil and vegetation in the area show markedly elevated manganese concentrations (*9*).

In addition to the generally increased manganese concentrations in blood from the aboriginal population, there is also a subset of people with very high blood manganese concentrations, approaching 1000 nmol/L. There appears to be an association between very high concentrations of manganese in blood, low serum iron, and the occurrence of a group of neurological disorders that have a prevalence of about 2% (*10*, *11*). The association has been reproducibly demonstrated, but at present it is not known whether these findings indicate a form of manganism affecting the community as a result of exposure to manganese in the environment.

The consistency of the increased values for blood manganese in the aboriginal community over a period of months indicates continued and uniform exposure to the metal. The group of aborigines showing symptoms of neurotoxicity and having very high values for blood manganese may have unusual or defective manganese regulatory processes.

We plan to apply the present analysis to detection of individuals at risk of accumulating a dangerous body burden of manganese and possibly suffering neurological insult.

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# ATTACHMENT C-6

Cawte et al. (1989)

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FORUM SECTION

# Motor Neurone Disease of the Western Pacific: Do the Foci Extend to Australia?

JOHN CAWTE[1], CHARLES KILBURN[2] AND MARK FLORENCE[3]

*[1]Personal Chair in Psychiatry, University of New South Wales, Prince Henry Hospital, Little Bay, Sydney; [2]Schools of Psychiatry and Paediatrics, University of New South Wales, [3]Division of Fuel Technology, CSIRO, Lucas Heights, New South Wales, Australia*

*ABSTRACT: Hyperendemic foci of motor neurone disease, and other neurodegenerative disorders, are located in the western Pacific area, in the Japanese of the Kii Peninsula of Honshu Island, the Chamorros of the Mariana Islands, the Auyu and Jakai of West New Guinea. It is suspected that there is a common aetiologic pathway from toxic metal and essential minerals in these three foci. A fourth focus of motor neurone disease occurs in an isolated tribal group living on the same Pacific longitude, at Angurugu on Groote Eylandt in the Gulf of Carpentaria, Northern Australia. This environment is also characterized by local ecological extremes, including low calcium and iron, and high manganese. The "Angurugu syndrome", described in this paper, shows dysfunction of motor neurones, including upper and lower motor, cerebellar, extrapyramidal and cranial nerves, especially oculomotor. About half the cases emerge in adult life. The others are evident in early childhood. The syndrome is viewed not as simple manganism but as manganism synergistic with accompanying mineral changes. No autopsy studies have been carried out. This paper suggests that this syndrome incurs a loss of the neurotransmitter dopamine. A case study is presented that indicates the unusual range of symptoms, including ataxia, and partial relief by L Dopa (Sinemet).*


**Key Words:** Motor Neurone Disease, Western Pacific, Neurodegenerative Disorders, "Angurugu Syndrome" Dopamine, Humans

## INTRODUCTION

Readers of *The Lancet* may recall reports in 1987 of the Angurugu syndrome, a range of neurological motor disorders affecting certain tribal Aborigines living on Groote Eylandt in the Gulf of Carpentaria in North Australia (1987). Its longitude is 138° East, as with other West Pacific motor disorders. It was first described, in 1980 by Kiloh (1980) and was reviewed in the book *Amyotrophic Lateral Sclerosis in Asia and Oceania*, in 1984.

Our attribution "synergistic" follows a suggestion by Shukla and Singhal (1983) who point out that the problem of metal toxicity is complex owing to simultaneous or successive exposure of a population to different physical,

Please send requests for reprints to Dr Mark Florence, CSIRO Centre for Advanced Analytical Chemistry, Private Mail Bag, Menai, N S W, AUSTRALIA 2234

[3]Presently Department of Health, Darwin, Northern Territory, Australia

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chemical, biological and psychological factors. The net toxic manifestations produced by multiple exposure should be different from those produced by a single factor, as the result of their additive, synergistic or antagonistic action. Even though the metal may not exist in sufficient amounts to cause any disability, toxicity could result when a second factor is also present, including the age of exposure.

On Groote Eylandt, synergistic factors are widespread. Four that we shall address are:

(1) Tribal Aboriginal genetic constitution and lifestyle;

(2) A familial sensitivity to manganese;

(3) A diet reduced in dopamine oxidation inhibitors, thiamine and ascorbic acid; and

(4) Local ecological effects such as low levels of calcium and iron.

These factors will be indicated, after prefatory comments on the "classical" features of manganism and the "distinctive" features of the Angurugu syndrome that render it synergistic as an "ethnic-geographic isolate".

## "CLASSICAL" FEATURES OF MANGANISM

Reports have been accumulating of the chemical toxicity of metals dispersed into the environment by natural as well as industrial sources. The removal of lead from petrol as an anti-knock agent has brought only temporary comfort, because organic compounds of manganese take its place, a matter being examined by the Canadian manganese researcher who assists our study, (John Donaldson, 1988). Special attention must be directed to the nervous system because relatively low manganese levels affect the action of the neurotransmitters, producing behavioral reactions of concern to neuropsychiatry. Manganism, as a collective term, tends to have an insidious onset and span of symptoms so broad as often to obscure recognition. Classical studies by workers such as (Rodier, 1955; Cotzias, 1974; Chandra, 1974) offer guidelines.

Manganism affects some of those who live in manganese polluted environments, such as manganese mines and their vicinity, or workers in the manganese-using industries of steel production and battery fabrication. There is a wide individual response to exposure such that only a small proportion develop symptoms of poisoning. This is analogous to the observation that only a minority of those eating excessive sugar develop diabetes, or salt, hypertension. Synergistic, additive or antagonistic factors play a part.

The symptoms of manganese poisoning may be divided into three phases of mild, moderate and severe. Mild poisoning (also called premanganism) shows symptoms of a psychiatric nature: mental instability, followed by complaints of lassitude and sexual impotence. Moderate intoxication shows symptoms of a neurological kind: clumsiness, speech disorders, difficulty in walking, and a change in facial expression. Chronic severe poisoning shows tremors, gait disturbances (described in some cases as "cock walk"), mask-like face, ataxia and other motor disturbances, with occasionally dementia.

Laboratory investigations are not specific in confirming the diagnosis of manganism. Normal or only slightly raised values of manganese may be found in the serum. Since manganese has a short half life in the blood, elevated levels do not indicate toxicity, only recent exposure. A gradual elevation of serum calcium as the disease progresses may be of value in diagnosis.

Clinical study at Groote Eylandt has logistic disadvantages. Some of the cases transferred to Prince Henry Hospital in Sydney have shown laboratory evidence of pathology. Failure to procure autopsy material is due to the remoteness of both the culture and the site. (Angurugu is a 500 kilometer return trip from Darwin, and a 2,000 kilometer return trip from Sydney). Agreements now exist with the community for a pathologist who has visited Groote Eylandt, Professor Bruce Warren, to examine diseased parts of the body after death. Autopsy samples can also be rapidly and non-destructively analysed by neutron activation analysis.

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## DISTINCTIVE FEATURES OF THE ANGURUGU SYNDROME

At Angurugu, the disease profile approximates that described for manganism in Egypt rather than for that in Chile. (El Naby and Hassanein, 1965) observe that chronic toxic manifestations among manganese miners in the Sinai desert point to involvement not only of the basal ganglia but frequently of other parts of the nervous system such as the pyramidal tracts and the cerebellum and its connexions. Angurugu patients exemplify this range, with a relative frequency of cerebellar symptoms, as well as cranial nerve involvement. A fatal case preceding our study, almost certainly suffered a bulbar palsy.

The Egyptian cohort showed more acute psychotic excitements than have other series, though the mental condition in Egyptian patients improved after removal from exposure for a few weeks. While mild psychiatric symptoms regressed, the neurological ones were stationary or showed progression. The present authors are unsure about the significance of acute excitements at Angurugu, because the language and cultural patterns are not easy to apprehend. Mild disturbances are reported by many patients and relatives, involving irritability and changes in mood and conduct. More Angurugu inhabitants are arrested by the police and removed from the locality for their distressed behaviour than from any other local group in Australia. This is commonly attributed to alcohol, but we have not studied whether pre-existing manganese absorption may contribute. Its common occurrence is evident from hair studies by Florence *et al.*, (1987).

Familial sensitivity is one feature in which Egyptian cases also resemble the Angurugu syndrome. While only a small percentage of Egyptian labourers developed toxic manifestations, as in other endemic centres, 15.5% of the cases were brothers or cousins. This familial sensitivity to exposure is dramatic at Angurugu and will be considered as one of the "synergisms". (Gajdusek, 1987) likens the Angurugu syndrome to the Madras motor neurone disease.

## FIRST SYNERGISM

### Genetics and Temperament

Of the four anthropoid divisions of the human race: Caucasoid, Negroid, Sinoid and Aboriginal, least is known of the biological nature of the latter small division. The cultural and personality variables so evident to the casual observer should not lead him or her to overlook the biological differences. Some genetic differences of Aborigines have relevance to health, for example the comparative length of limbs to body, the absence of myopia, and a high insulin resistance associated in modern culture contact with high levels of type 2 diabetes and obesity. Genetic differences shown by blood groups and fingerprints were elaborated in 1972. They have not yet been examined for either those affected or not affected by the Angurugu syndrome. Genetic disorders of trace element transport, known in humans and other animals, have been detailed by (Danks, 1985). Copper accumulation, as an inborn error of trace element metabolism causing damage in the kidneys and brain in Wilson's disease, is probably the best known instance. Little is known of manganese in such matters, or whether the Angurugu syndrome represents an example.

Basic temperamental differences may be genetically related. Aborigines live more comfortably "close to the earth". At Angurugu they cook their dampers in the earth, and see no need to wash earth from their skin surface each day as Europeans do. Unshod feet make them susceptible to suffer low haemoglobin values. Aborigines are much more a part of the earth and the constituents it may proffer, such as manganese.

## SECOND SYNERGISM

### Familial Sensitivity to Manganese

Like the affected Egyptian desert dwellers, the cases at Angurugu come from identified families. Two extended clans provide most of

TABLE 1. Inhibition of Manganese-Catalyzed Oxidation of Dopamine ($3 \times 10^{-4}$M).

| Inhibitor | Concentration (M) | Reduction in Oxidation Rate (%) |
|---|---|---|
| Ascorbic acid | $5 \times 10^{-5}$ | 100 |
| Dehydroascorbic acid | $1 \times 10^{-3}$ | 100 |
| Thiamine | $1 \times 10^{-3}$ | 100 |
| Folic acid | $1 \times 10^{-3}$ | 74 |
| Biotinc | $1 \times 10^{-3}$ | 53 |
| Pyridoxine | $1 \times 10^{-3}$ | 44 |

the cases. The Canadian anthropological authority, (Professor David Turner, 1987) notes: "The two clans most affected by the disease - the Lalara and the Bara - never intermarry and have not done so within genealogical memory. Neither are indigenous to Groote, the Bara originating on Bickerton Island (nearer to the mainland) and the Lalara on Bickerton and before that on the adjacent mainland. Having taken up land in the north-western and central western parts of Groote Eylandt respectively when they arrived here, however, they have been in the most prolonged contact with the most manganese-afflicted parts of the island".

It would therefore be incorrect to attribute the syndrome to interbreeding, as some observers have. It has a familial pattern, in two different clans. We find three affected brothers in one family, and two affected sisters in another. Each sibship has normal siblings. Our numbers are not sufficient to decide whether we are dealing with a Mendelian dominant or recessive sensitivity to environmental manganese, though we may suspect the former, as with Wilson's disease involving copper.

## THIRD SYNERGISM

### Diet Commonly Deficient in Dopamine Oxidation Inhibitors, Thiamine and Ascorbic Acid

Melbourne researchers, O'Dea *et al.*, (1988) have shown how Aborigines develop a high frequency of type-2 diabetes mellitus and obesity when they make the change from "bush tucker" to "store tucker". Most of the tribal residents at Angurugu, making this transition, prefer a diet in which the basic elements are refined food, including the staples of wheat flour (not enriched with vitamin additive in Australia as in most modern countries) cane sugar and honey. There is limited use of fresh fruit and vegetables. It seemed possible to us that a vitamin-low diet of this kind could be a synergistic factor.

In our research laboratory at C.S.I.R.O., Sydney, we investigated manganese catalysis of dopamine oxidation by oxygen. Detailed *in vitro* kinetic measurements showed that the oxidation proceeds via a manganese (II) / (III) redox couple, the superoxide radical, and a semiquinone intermediate, with a pseudo first order rate constant of $1.5 \times 10^{-2}$ min $^{-1}$ at pH 7.7 (phosphate buffer) and 37° C.

The neurotoxicity of manganese results from the depletion of dopamine and the production of the neurotoxins, dopamine quinone and hydrogen peroxide. In addition, a side reaction from dopamine oxidation produces significant concentrations of hydroxyl radical ($OH^{\bullet}$), the most energetic chemical species known, and which is highly damaging to biological systems.

A wide range of compounds was tested *in vitro* (pH 7.5-8.0, 0.02 M phosphate buffer) for their ability to inhibit dopamine oxidation. Few of the compounds tested gave a signifi-

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npounds was tested *in* vl phosphate buffer) for dopamine oxidation. tested gave a signifi- cant reduction in oxidation rate, and all those that did were positively charged (e.g., thiamine) or had a positively-charged group in the molecule (e.g., ascorbic acid).

Ascorbic acid (vitamin C) and thiamine (vitamin B1) were the most effective inhibitors, completely inhibiting oxidation both in the presence and absence of manganese (Table 1). Thiamine-neurotransmitter interaction has been observed in humans by (Freye and Hartung, 1982). The inhibiting effect of dehydroascorbic acid shows that the mechanism is not simply chemical reduction, although ascorbic acid was able to reduce dopamine quinone back to dopamine. The effectiveness of positively-charged molecules suggests that inhibition involves stabilization of the negatively-charged semiquinone intermediate, thus quenching the oxidation chain reaction.

Dopamine loss is affirmed by (Donaldson and Barbeau, 1985). Since many of the Groote Eylandt Aborigines are likely to be deficient in ascorbic acid (poor diet) and thiamine (alcohol intake), it is possible that depletion of these vitamins exacerbates the disease. To test this theory, a first step would be to measure the vitamin status of all aborigines identified as being affected by the Angurugu Syndrome, plus a group of controls. If deficiences were found, an intervention program with supplementation of vitamins C and B1 should be considered. These vitamins are cheap and, even in large excess, are completely non-toxic.

Confirmation by (Archibald and Tyree, 1987) that trivalent manganese can attack catecholamines brings a freshness of outlook to clinicians studying the problem that manganese can be an insidious environmental toxin, at least in part because of its capability of valency change under varied conditions.

## FOURTH SYNERGISM

### Additive Effects of Mineral Extreme

Other factors able to shape the outcome of manganese exposure should be indicated. There is a range of them in the unusual geology of Angurugu. Alkali earths with calcium deficiency is the outstanding one.

Six samples of Groote Eylandt water were sent by our colleague Dr. John Hargrave in the Northern Territory Health Department to Professor R. Garruto's laboratory in Bethesda, MD. in 1983. Reporting on 31 elements, (Garruto , 1983) drew attention to remarkably low levels of calcium and magnesium. Subsequent tests by the government water supply chemists and by the mining company confirm very low levels of calcium and probably of magnesium and zinc. Manganese can displace calcium from nerve endings and hence disrupt conduction. This is more likely to happen in individuals with high manganese and low calcium status.

The indigenes were protected against calcium deficiency during their days of active fishing, as did the Chamorro of Guam, where a similar geological profile apparently applies. Fishing fell into relative decline during the early days of white settlement. "Government Rations" of unfortified white flour, rice, sugar, tobacco and tea do not offer a good source of calcium or other missing minerals. Proper dietary supplementation presents difficulties. Many Aboriginal people have a distaste for food containing cow's milk because of their lactose intolerance.

Local mineralogical extremes must raise the concern of the clinician. Why does the Western Pacific region show sites of low calcium and possibly other minerals, in local areas? It seems unlikely to derive from climatic causes such as heavy monsoonal rain with elution of soluble mineral substances. There may exist a unique combination of surface geochemical partitioning, analogous perhaps to the iodine deficiency areas of potential goitre in mountain zones. The theoretical significance of low environment calcium, with diminished food intake was shown by the seminal studies of the Japanese Yase (1972). Stimulated by these studies, Garruto *et al.*, and (Garruto, 1985) studied the elemental profile of the soil and water in the Western Pacific foci of motor neurone disease, confirming the effect of unusually low concentrations of calcium and magnesium. The longitude bearing of Groote Eylandt of East 138° is the same as the other Western

Pacific neurological complexes.

Other regional public health issues have to be considered on Groote Eylandt. The potentially poisonous effect of non-eluted cycad nuts can be ruled out, according to our observations. Many people of the Aboriginal community suffer from ankylostomiasis. Iron deficiency from the widespread occurrence of hookworm anaemia can be an additive factor. Iron and manganese have a similar uptake mechanism; anaemic persons have enhanced absorption of both iron and manganese, and are known to be more susceptible to the toxic effects of this element, as (Cotzias, 1968) showed. Boiled "billy tea" is a good source of manganese, and its tannin content also reduces the intake of iron in the diet. Curiously enough, nicotine may act as a prophylactic towards neurotransmitter loss diseases such as Parkinson's, acting as a free radical acceptor and an inhibitor of dopamine oxidation (1983, 1981). Smoking is widespread in Groote Eylandt Aborigines. Finally, chronic infections and high alcohol intake, both commonplace at Angurugu, may be factors that exacerbate manganese toxicity.

## RECOMMENDATIONS

The Angurugu syndrome of diversified motor disorders affecting the nervous system seems best explained as a synergistic manganism with dopamine loss. Synergistic or additive factors that possibly contribute are exemplified. From the point of view of the affected community, it seems desirable that the families chiefly affected should consider relocation away from the manganic outcrop upon which they live. Possibly a site of relocation, most contiguous to "home", would be the mining company town and port of Alyangula, 20km to the north.

Other community members who absorb manganese, as shown by clinical tests of scalp hair, blood, or urine, should be assisted by the local health centre in minimizing the exacerbating or triggering factors that have been discussed. This would require the services of a resident medical officer. Indeed the Angurugu Community Council has offered to pay half the salary of a doctor to serve them, if one can be found.

The high ambience of manganese and low concentration of calcium seem to be interactive features, leading to dopamine oxidation with production of neurotoxic substances. Complimentary with this hypothesis are our *in vitro* reports of compounds that inhibit the mananese-catalysed oxidation of dopamine. As with Wilson's disease, it is likely that a genetic factor is involved, perhaps as a familial sensitivity to manganese. An imbalance of elevated metal (manganese) and low salts (calcium and magnesium, possibly) contribute. Our data support the hypothesis that an ambient defect in mineral metabolism, facilitates absorption of potentially toxic metals such as manganese. Subsequent correction of appropriate minerals then cannot prevent or reverse the neurone damage that has occurred.

While the Angurugu people have our first consideration, the reactions to this situation by white lobbies observing it have been highly significant and will be indicated elsewhere.

## ACKNOWLEDGMENTS

At the Darwin conference on these disorders held in June 1987, Professor John Mathews, Director of the Menzies School of Health Research, accepted the role of director of future research. Although he has not been associated with the findings presented in this paper, the writers are appreciative of his support and hospitality in Darwin. Our chief consultant on the properties of manganese is Dr. John Donaldson, Senior Consultant of BioTox Research Services of Pointe Claire, Quebec, Canada. Dr. Donaldson has visited in Sydney and Groote Eylandt, and has provided information by correspondence. He is the author of the chapter on Manganese in Human Health forthcoming in the document: *Manganese in the Canadian Environment*, by the National Research Council of Canada through its Associate Committee on Scientific Criteria for Environmental Quality.

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## EDGMENTS

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among a traditionally-oriented Aboriginal population possessing limited English vocabulary or widespread grasp of public health issues, calls for special thanks for the co-operation which has come willingly from Angurugu. The Chairman of the Angurugu Community Council, Mr. Murabuda Wuramarba and the Council members at Angurugu have given us warm hospitality, and we are grateful for the personal help of one of the neurologically affected victims, Mr. Kevin Lalara. The Anglican Church Missionary Society, acting through the social workers Ms. Mary Harris (Mrs. S. Eves), and the chief clerk at Angurugu community, Mr. Lance Tremlett and his wife Gwen, has been invaluable.

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The National Health and Medical Research Council (N.H. & M.R.C.) provided the first author with the award enabling the second author to work in the field for two years, examining the neurological patients and assisting sick members of the community in general. Dr. Gordon White, of Canberra, shared in discussions of the work on behalf of N.H. & M.R.C.

At home in Sydney, many colleagues have been helpful, especially Emeritus Professor Leslie Kiloh, from neuropsychiatry, and Dr. Keith Lethlean, from neurology. We are indebted to the secretary looking after this project at Prince Henry Hospital, Ms. Wilma Sturgeon, for her administration and office procedures.

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ATTACHMENT C-7

Review of

*Epidemiological Survey Among Workers Exposed to Manganese: Effects of Lung, Central Nervous System, and Some Biological Indices* by Roels et al., American Journal of Industrial Medicine: Vol. 11, 1987, pp. 307-327.

Emanuel Landau, Ph.D.

This cross-sectional epidemiological study of 141 male workers exposed to moderate levels of airborne manganese was published in two sections. The first article discussed the relationship between external and internal parameters of exposure of these workers. The second article, which is being reviewed, discusses respiratory illness, lung function tests, symptoms related to the nervous system, the neurological examination and the psychomotor tests. The first section was not made available so that possible questions regarding exposure can't properly be raised. Thus, the extent of job transfer and constancy of exposure are not known.

The concentration of manganese was measured in blood and urine. Hair and nail specimens were not obtained from the subjects or controls. This is surprising since the article notes the lack of "good biological indicators of Mn exposure."

The study design provided for two industrial populations; one, the manganese factory; the other, a nearby chemical plant. Which chemicals the chemical plant workers were exposed to is not stated. Also, there is no indication of the stability of that labor force.

The matching process provided for matching by socio-economic status and "background environmental factors". No information is provided on what these "background environmental factors" include.

The article is careful to point out that some of the tests were "blind"; possibly all were. However, in occupational health studies with which I have been involved, to avoid identifying the site of employment, the subjects were asked not to give that information to the investigators. Also, the data sheet did not indicate to the examiners the possibility of exposure. Obviously, the possibility of examiner bias is to be minimized.

There is missing text between pages 312 and 313 and again between pages 319 and 320. Page 313 notes that "coffee and beer consumption" was comparable between the controls and Mn-exposed group and there was no statistically significant difference between the number of workers in both groups consuming these beverages. Nonetheless, despite the lack of statistical significance, isn't the 80% proportion of beer drinkers among the Mn-exposed as compared with the 70% among the controls of significance in analyzing the test results? Also, the greater number of glasses of beer per day (both median and mean) for Mn-exposed workers, even with a lesser number of years of consumption, lead to difference of beer consumption of approximately 3% between Mn-exposed and control workers. Shouldn't the analysis have considered not only exposure to manganese but, also, extent of beer consumption?

The treatment of smoking is disturbing. Why combine current and ex-smokers? Table 2 would appear to indicate that manganese workers who were ex-smokers may have quit smoking for health reasons. Their average of 30.4 cigarettes may be compared with 20.6 for ex-smokers who are controls and 17.1 for manganese workers who are current smokers.

I regret that I do not understand the derivation of the integrated cigarette smoking index. Nonetheless, the statement that the integrated cigarette smoking index is higher in the control group (page 314) doesn't appear to be true for ex-smokers. If this is indeed the case, then the assertion that "Mn exposure is the likely cause of higher prevalences of respiratory tract symptoms in the Mn group" may represent an overstatement.

I am disturbed by the lack of supporting data for conclusions. Thus, dusty atmospheres as related to prevalence of respiratory tract symptoms is discounted as are lung function differences in current versus ex-smokers among manganese workers. Again, why were the subjective symptoms relating to the nervous system, including the four specifically noted, not examined by smoking status. One may also ask why smoking as a variable was not considered for the other central nervous system tests.

Since we are not aware of the actual conduct of the study, we do not know if the manganese workers knew they were the object of the study. Further, were these workers made aware of the adverse effects ascribed to manganese exposure? Thus for example, is it possible that the reports of impotence by six manganese workers, as noted on page 323, could have arisen from the publicity about the study and the manganese workers knowing of presumed effects?

The uncritical acceptance of a possible selection bias, that moderate manganese exposure may have prompted the most susceptible workers to find other employment, may represent nothing more than conjecture. Was any effort made to find out why manganese workers left the plant? I was also disturbed by the uncritical acceptance of other research which is apparently less than adequate. One illustration is the 1973 study of school children by Nogawa et al.

Despite these reservations, the summary statement that "this study demonstrates that time-weighted average exposure to manganese dust (total dust) of about 1 $mg/m^3$ may still lead to the occurrence of preclinical adverse effects in the lungs and the central nervous system in some workers exposed for less than 20 years" appears tenable.

# ATTACHMENT C-8

Wang et al. (1989)

*British Journal of Industrial Medicine* 1989;46:856–859

# Manganese induced parkinsonism: an outbreak due to an unrepaired ventilation control system in a ferromanganese smelter

J-D WANG,[1] C-C HUANG,[2] Y-H HWANG,[1] J-R CHIANG,[1] J-M LIN,[1] J-S CHEN[1]

*From the Department of Internal Medicine and Clinical Pathology, National Taiwan University Hospital, and Center for the Research of Environmental and Occupational Diseases,[1] Graduate Institute of Public Health, National Taiwan University College of Medicine, and Department of Neurology,[2] Chang-Gung Memorial Hospital, Taipei, Taiwan*

ABSTRACT Several cases of parkinsonism were found in a ferromanganese smelter after the ventilation system had broken down and had not been repaired for eight months in 1985. To determine the aetiology and prevalence of parkinsonism, 132 workers were submitted to thorough medical examination and estimated air concentrations of carbon monoxide and manganese at different worksites. Only six of eight workers performing electrode fixation or welding during 1985 developed parkinsonism. They were exposed for 30 minutes each day, seven days a week, to high concentrations of air manganese (> 28·8 mg/m$^3$). There was a consistent trend between the index of exposure to manganese and signs and symptoms exhibited by extrapyramidal systems. After repair of the ventilation system, the air concentration of manganese during electrode fixation and welding decreased to less than 4·4 mg/m$^3$; furthermore, no new cases of parkinsonism have been observed. Workers with parkinsonism recovered partially after removal from original worksites and treatment with levodopa. It is concluded that the outbreak resulted from exposure to high concentrations of manganese fumes through the breakdown of the ventilation system.

In October 1985 a 44 year old ferromanganese smelting worker attended the Chang-Gung Memorial Hospital department of neurology complaining primarily of spastic gait. He had a mask like face, a reduced rate of blinking, clumsiness, micrographia, and bradykinesia. Parkinsonism was diagnosed. Because several other coworkers exhibited similar symptoms, and parkinsonism has been reported to be associated with exposure to manganese,[1–4] we decided to study the aetiology and prevalence of parkinsonism among workers at this factory.

## Material and methods

The factory has been operating for 18 years and consists of three major departments: the ferromanganese smelting, foundry, and management office. During 1983, the ventilation systems (particularly the air cleaning device) of the three furnaces of the smelting department were not in good working order. The owner ordered a new system and removed the old one in December 1984 but did not discontinue smelting operations. Owing to delays in the installation of the new equipment, workers were subsequently exposed to raised concentrations of manganese. Workers were classified into four groups according to the probability of exposure to manganese at their occupations: degree 0, office, designing, and packaging workers; degree 1, foundry, foundry related, non-furnace maintenance workers, and metal cutters; degree 2, furnacemen; and degree 3, furnace foremen and maintenance workers. Air samples were collected using personal and area samplers. Manganese concentrations were estimated by graphite furnace atomic absorption spectrometry,[5] and carbon monoxide concentrations by direct reading from a Kitagawa COM-4 carbon monoxide analyser.

Each of the 132 workers in the factory underwent a

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comprehensive physical examination that included detailed occupational and medical histories, chest radiography, electrocardiography, liver function tests, blood creatinine assays, and complete blood count. Then 123 were also interviewed for neurological symptoms; blood samples were taken from 68 of these workers to estimate the manganese in whole blood.[10] The association between exposure to manganese and each neurological symptom was calculated using the chi-squared test for trend.[11]

## Results

An area air sample taken about 3·5 m from the top of the furnace, where degree 3 workers operate on the electrode, contained 28·8 mg/m³ of manganese, whereas two samples taken near the side of the furnace contained 1·0 mg/m³. According to our field observations, workers who operate on the electrode were probably exposed to manganese fumes higher than 28·8 mg/m³ manganese for at least 30 minutes a day, seven days a week. The air concentration of carbon monoxide was less than 15–60 ppm both near the side and near the top of the furnace.

All furnacemen were men, most of whom had been employed for more than 10 years (table 1). Six of the eight degree 3 workers developed parkinsonism, as diagnosed by standard neurological examination. No cases were found in the other workers. Furnacemen (degree 2) were exposed to 0·5–1·5 mg/m³ manganese, whereas foundry workers who worked in a separate building were exposed to only 0·1 mg/m³. We did not measure manganese concentrations in the management office but exposure was probably even less. Table 2 shows that the blood manganese concentration increased with exposure. Table 3 shows that the frequency of neurological symptoms and extrapyramidal signs increased with the degree of exposure to manganese and supports the possibility of some early stage cases of parkinsonism. No similar association was found between the degree of exposure and the results of liver function tests, blood creatinine assays, electrocardiograms, and chest radiographs.

## Discussion

Parkinsonism is a symptom complex consisting of bradykinesia, rigidity, tremors, and impaired postural reflexes. Its aetiology includes infections, toxins, pharmacological causes, and other degenerative diseases.[12,13] That manganese played a causative part in the six cases of parkinsonism among the ferromanganese workers is supported by several observations. All six were below the usual age of onset of ideopathic Parkinson's disease[12,13]; two were under 47 and the other four under 40. All six worked in the same occupational setting, where the manganese concentration usually exceeded 28·8 mg/m³, and all but one showed raised blood manganese concentrations (greater than 20 μg/l). The blood sample of the one patient with 10 μg/l manganese had been taken six months after he had left the company.

Furthermore, it is unlikely that these cases were the result of exposure to other causal agents. The air concentration of carbon monoxide was usually below 60 ppm, which normally does not produce a carboxyhaemoglobin concentration higher than 10%[14]; carboxyhaemoglobin concentrations less than 20% are

Table 1 *General characteristics of workers with different levels of manganese (Mn) exposure*

| | *Index of exposure to Mn* | | | |
|---|---|---|---|---|
| | *0 (n=32)* | *1 (n=68)* | *2 (n=24)* | *3 (n=8)* |
| % Male | 53·1 | 95·6 | 100 | 100 |
| Age (y) | 34·4 ± 9·64 | 39·7 ± 10·74 | 46·5 ± 8·3 | 40·1 ± 4·2 |
| Months spent at working (current job or factory) | 85 ± 67 | 85 ± 64 | 134 ± 74 | 114 ± 61 |
| % Smokers | 38 | 59 | 67 | 88 |

Table 2 *Concentration of blood manganese (Mn) among workers with different exposure categories*

| | *Index of exposure to Mn* | | | |
|---|---|---|---|---|
| | *0 (n=17)* | *1 (n=28)* | *2 (n=16)* | *3 (n=5)* |
| Mn conc of blood (μg l) ± 1 SD: | 14·9 ± 9·2 | 25·2 ± 8·6 | 31·3 ± 15·6 | 146 ± 155 |
| Median | 12·0 | 22·5 | 28·3 | 80 |
| Range | 3–35 | 13–38 | 13–82 | 10–405 |
| Haemoglobin concentration (mg 100 ml) ± 1 SD: | 14·8 ± 1·5 | 16·1 ± 1·3 | 15·8 ± 1·4 | 15·5 ± 1·0 |
| Median | 14·7 | 16·2 | 15·8 | 15·3 |
| Albumin globulin ratio ± 1 SD: | 1·4 ± 0·1 | 1·4 ± 0·2 | 1·4 ± 0·1 | 1·6 ± 0·1 |
| Median | 1·4 | 1·4 | 1·4 | 1·6 |

Table 3 *Prevalence of neurological signs and symptoms among workers stratified by different levels of exposure to manganese (Mn)*

| Symptoms and signs | No of workers at different indices of exposure to Mn | | | | |
|---|---|---|---|---|---|
| | 0 (n=32) | 1 (n=64) | 2 (n=19) | 3 (n=8) | p* |
| Signs: | | | | | |
| Bradykinesia | 0 | 0 | 0 | 6 | <0·0001 |
| Rigidity | 0 | 0 | 0 | 6 | <0·0001 |
| Gait abnormality: | | | | | |
| Unable to walk backward | 0 | 0 | 0 | 6 | <0·0001 |
| Stopping while turning around | 0 | 0 | 0 | 5 | <0·0001 |
| Stuttering | 0 | 0 | 0 | 5 | <0·0001 |
| Tremor | 0 | 1 | 0 | 4 | <0·0001 |
| Symptoms: | | | | | |
| Weakness | 4 | 4 | 9 | 7 | <0·0001 |
| Cramps in arms and legs | 2 | 7 | 5 | 4 | 0·0012 |
| Loss of libido | 1 | 5 | 4 | 3 | 0·002 |
| Dislike of talking | 2 | 1 | 1 | 4 | 0·0026 |
| Unable to perform delicate job | 1 | 4 | 3 | 3 | 0·003 |
| Hallucination | 0 | 1 | 0 | 2 | 0·0054 |
| Fatigue | 8 | 15 | 11 | 4 | 0·017 |
| Anorexia | 2 | 7 | 5 | 2 | 0·032 |
| Fatigue, heaviness in legs | 4 | 6 | 4 | 4 | 0·042 |
| Hypersalivation | 2 | 3 | 1 | 3 | 0·046 |
| Muscle cramping pain | 2 | 7 | 4 | 2 | 0·06 |
| Insomnia | 2 | 9 | 4 | 2 | 0·08 |
| Drowsiness | 8 | 5 | 4 | 0 | 0·16 |
| Thirst | 6 | 15 | 3 | 1 | 0·67 |
| Abdominal cramps | 0 | 6 | 1 | 0 | 0·70 |
| Headache | 6 | 7 | 5 | 1 | 0·87 |
| Irritability | 6 | 7 | 4 | 1 | 0·90 |

*Chi-squared test for trend.

not believed to result in parkinsonism.[15] In addition, no incidents of carbon monoxide poisoning resulting in parkinsonism sequelae[16] had occurred in the factory since its establishment in 1967. Furthermore, no evidence of other medical problems, such as infections of the central nervous system, tumours, hypoxia, or Wilson's disease, was observed in these six workers. Finally, all six cases were diagnosed between August 1985 and May 1986 after the breakdown of the ventilation system (December 1984); no cases had been diagnosed before then, nor have any new cases been diagnosed since the installation of a new system in August 1986. Because the only cases of parkinsonism in the factory's 20 year history developed during the short period of 20 months when the ventilation system was not operating properly, we conclude that manganism may be attributed to the faulty and removed ventilation system, which resulted in daily worker exposure to high concentrations of manganese fumes.

The poor correlation found between blood concentration of manganese and duration of employment is in accordance with Tsalev *et al.*[10] At the same time, we did find a clear relation between the level of manganese exposure and the blood concentration of manganese, as indicated in table 2. In fact, only four of 17 employees in the degree 0 exposure group had a blood manganese concentration above 20 μg l; this is partially explained by the fact that these four workers were occasionally required to enter high exposure work areas. These results concur with data from rats and monkeys[17] and from other manganese workers.[18] Blood manganese concentration may therefore be used as an indicator of current environmental exposure but not of chronic exposure.

Although some authors have tried to explain individual susceptibility to manganese as the result of nutritional deficiency and variation in absorption efficiency due to conditions such as anaemia[19] and low albumin globulin ratios,[20] we did not find any evidence of abnormal concentrations or albumin-globulin ratios among the cases with parkinsonism when compared with degree 2 workers or other (non-parkinsonism) degree 3 workers.

On completion of the epidemiological study, the factory owner responded and took action to enhance dilutional ventilation and implement a new system of local ventilation. Subsequently, air manganese concentrations were usually below 1·6–2·1 mg m$^{-3}$, with short term concentrations of around 2·9 mg m$^{-3}$ during the electrode welding operation; the foremen were exposed to 4·4 mg m$^{-3}$ manganese while they operated on the electrodes. There have been no new cases of parkinsonism since implementation of the protective measures. The workers with parkinsonism were removed from their original jobs and treated with

levodopa, which appeared to improve the neurological symptoms by 50%, as measured by the modified Columbia Scales.[21]

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# ATTACHMENT C-9

Huang et al. (1989)

# Chronic Manganese Intoxication

Chin-Chang Huang, MD; Nai-Shin Chu, MD; Chin-Song Lu, MD; Jung-Der Wang, MD; Jin-Lian Tsai, MSc; Jia-Liang Tzeng; Erik C. Wolters, MD; Donald B. Calne, MD

• **We report six cases of chronic manganese intoxication in workers at a ferromanganese factory in Taiwan. Diagnosis was confirmed by assessing increased manganese concentrations in the blood, scalp, and pubic hair. In addition, increased manganese levels in the environmental air were established. The patients showed a bradykinetic-rigid syndrome indistinguishable from Parkinson's disease that responded to treatment with levodopa.**

(*Arch Neurol.* 1989;46:1104-1106)

Manganese is used in metal alloys, in the manufacture of chlorine gas, dry battery cells, paints, varnish, enamel, and linoleum, in the process of coloring glass and soaps, and as an antiknock agent in lead-free gasoline. Exposure in mining and industry has resulted in chronic manganese poisoning, first reported in 1837,[1] in Chile, Morocco, Cuba, India, Japan, the USSR, and the United States.[2]

Manganese ore enters the body by inhalation of the dust and by swallowing the particles, mainly containing manganese dioxide. Neuropathologic changes, reported so far in only 10 autopsy cases, are characterized by degeneration of the medial segment of the pallidum, often of the caudate nucleus and the putamen, and rarely of the substantia nigra.[3]

Clinical signs generally appear after exposure for 6 months or longer. In manganese miners, but not manufacturers, mild and mostly transient psychomotor disturbances such as hallucinations, emotional instability, and compulsive and aberrant behavior (so-called manganese madness) occur early in the clinical course and last about 3 months. Marked asthenia and extrapyramidal symptoms, such as hypokinesia, rigidity, and tremor, are the hallmarks in chronic intoxication. Other findings include dystonia, sialorrhea, sweating, impotence, and insomnia. The symptoms progress as long as exposure continues, and recovery is rare once the advanced syndrome develops; however, some improvement may occur.[4]

The underlying mechanisms whereby the inhalation of manganese dust results in disease are completely unknown. Hypotheses concerning the toxicity of manganese include (1) direct dopaminergic toxicity, (2) increased production of toxic free radicals, (3) decrease of oxidation-reduction mechanisms in the substantia nigra, (4) production of 6-hydroxydopamine or other toxic catecholamines with a decrease in protective thiols, and (5) oxidation or increase of auto-oxidation of dopamine with production of cytotoxic quinones.[5-7]

Levodopa, as well as edetate disodium calcium, provides some relief, but their therapeutic benefit is controversial.[8-10]

## PATIENTS AND METHODS

In October 1985, we suspected manganese-induced parkinsonism in a 44-year-old man working in a ferromanganese alloy factory. Clinical investigations in coworkers uncovered five more cases, all working in the same manganese smelting department on the third floor of this factory. All were men, between the ages of 35 and 45 years. All the patients were carefully screened for neurological, neurophysiological, and neuropsychological abnormalities. The neuropsychological tests included temporal orientation, orientation to personal information and place, serial digit learning, word sequence learning, judgment of line orientation, three-dimensional block construction, facial recognition, visual form discrimination,[11] line cancellation, visual naming, and Token test.

Environmental studies were performed and included the assessment of manganese air concentration, which was determined by graphite furnace atomic absorption spectrophotometry, and carbon monoxide concentration, determined by direct reading from a carbon monoxide analyzer (Riken Keiki Kogawa COM-4) in different air samples.

Manganese concentrations in blood, as well as in scalp and pubic hair,[12] were measured after a neutron flux of $2 \times 10^{13}$ neutrons per square centimeter second irradiation for 5 minutes, with a Ge(Li) detector coupled to a 4096-channel, pulse-height analyzer.

Therapeutic effects of both levodopa carbidopa and edetate disodium calcium in the patients were evaluated weekly with Modified Columbia Rating Scale assessments,[13] in which 0 indicated normal and maximum in deficit for each clinical picture as listed in Table 1, each form of treatment being given for 8 weeks in an open setting.

## RESULTS

Clinical examination of these six patients revealed bradykinesia with masked face, diminished blinking,

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From the Departments of Neurology, Chang Gung Medical College Hospital (Drs Huang, Chu, and Lu) and National Taiwan University Hospital (Dr Wang), School of Technology, Kaohsiung Medical College (Ms Tsai), and Institute of Nuclear Science, National Tsing-Hua University (Mr Tzeng), Taipei, Taiwan, Republic of China; and the Department of Medicine, Division of Neurology, University of British Columbia, Vancouver (Drs Wolters and Calne).

Reprint requests to Department of Neurology, Chang Gung Medical College, Chang Gung Memorial Hospital, 199 Tung Hwa North Rd, Taipei 10591, Taiwan, Republic of China (Dr Huang).

clumsiness with impaired dexterity, and gait abnormalities. All patients also showed rigidity; five had hypophonia, and three a mild, low-amplitude tremor and micrographia (Table 1). Impotence and insomnia were present in three patients. Only one patient showed personality changes. Electroencephalography, electromyography, nerve conduction velocity studies, and evoked potentials were all within normal limits. Neuropsychological evaluation revealed impairment in the facial recognition test and slowing in visual constructive praxis. Extensive blood tests, urinalysis, and computed tomography of the brain did not reveal any abnormalities. Manganese concentration in blood, scalp, and pubic hair in all cases indicated definite acute and chronic intoxication (Table 2); values ranged from 3 to 300 times the normal levels.

Environmental investigations showed raised manganese concentrations in the air, related to the distance to the smelting furnace, and probably caused by a defect in ventilation. Highest levels were about 28 mg/m³ (threshold limited value,[16] 1 mg/m³). All patients worked for more than 2 years in the direct vicinity of the furnace without any protective equipment, as did four other unaffected coworkers. Carbon monoxide concentrations were measured between 15 and 60 ppm (threshold limited value,[17] 50 ppm).

Six patients were treated with three

Table 1.—Survey of Clinical Symptoms in Chronic Manganese-Intoxicated Patients (Modified Columbia Rating Scale)

| Clinical Characteristic | Patient No./Age, y | | | | | |
|---|---|---|---|---|---|---|
| | 1/45 | 2/35 | 3/47 | 4/38 | 5/39 | 6/39 |
| Facial expression | 3 | 3 | 2 | 1 | 2 | 1 |
| Seborrhea | 1 | 2 | 2 | 1 | 1 | 1 |
| Salivation | 0 | 0 | 0 | 0 | 0 | 0 |
| Speech | 3 | 3 | 2 | 1 | 1 | 0 |
| Tremor | 0 | 0 | 0 | 1 | 1 | 1 |
| Rigidity | 2 | 2 | 2 | 1 | 2 | 1 |
| Dexterity | 3 | 2 | 2 | 1 | 1 | 2 |
| Rapid alternating movements | 2 | 2 | 1 | 1 | 1 | 1 |
| Foot tapping | 2 | 2 | 1 | 0 | 0 | 1 |
| Posture | 1 | 1 | 1 | 1 | 1 | 1 |
| Stability | 1 | 1 | 1 | 1 | 1 | 1 |
| Gait | 1 | 1 | 1 | 1 | 1 | 2 |
| Bradykinesia | 2 | 1 | 1 | 1 | 1 | 1 |

Table 2.—Manganese Concentration in Blood, Scalp, and Pubic Hair

| Patient No. | Manganese Concentration | | |
|---|---|---|---|
| | Blood, ppb | Scalp Hair, ppm | Pubic Hair, ppm |
| 1 | 101.9 | 30.2 | 55.7 |
| 2 | 159.5 | ... | 243.2 |
| 3 | 405.2 | 445.2 | 178.0 |
| 4 | ... | 27.7 | 26.7 |
| 5 | 383.7 | 59.3 | 318.3 |
| 6 | ... | 220.1 | 2,735.5 |
| Reference range | 7-12 | 0.1-2.2 | 0.3-9.8 |

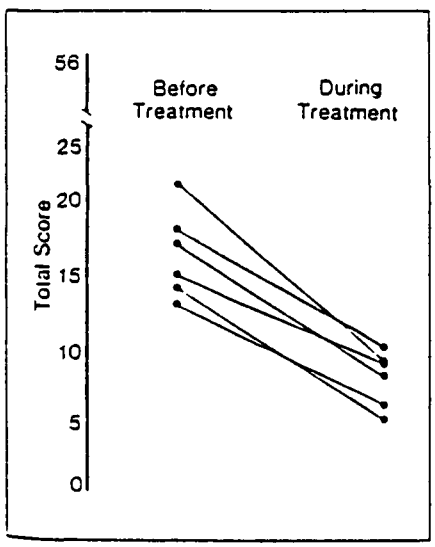


Fig 1.—Total score of the Modified Columbia Rating Scale for each patient derived from assessment of all clinical features before and during levodopa-carbidopa treatment in six patients with manganese intoxication.


Fig 2.—Facial expression in first patient before (A) and during (B) levodopa-carbidopa therapy

to six tablets combining 100 mg of levodopa with 25 mg of carbidopa daily in an open study for 8 weeks. Modified Columbia Rating Scale scores, measured each week, showed improvements over 50% (Fig 1). The improvements were statistically significant ($P < .001$, paired $t$ test). Figure 2 shows the effect of this treatment on facial expression in one of the patients. However, the facial recognition and visual constructive tests failed to show any improvement after the treatment. Edetate disodium calcium treatment in three patients over a similar period yielded no significant improvement.

## COMMENT

We have described six patients with manganese poisoning verified by finding increased manganese concentrations in the blood, indicating current intoxication, and in scalp and pubic hair, indicating chronic intoxication.[14] To our knowledge, the assessment of manganese levels in pubic hair has not been described before and seems to be useful; pubic hair levels were generally higher than scalp hair. Higher pubic hair values are probably related to the slower rate of growth of these hairs compared with scalp hair. Carbon monoxide concentrations in the working environment were also increased, but carboxyhemoglobin levels were not significantly raised nor were there any clinical signs or symptoms of carbon monoxide intoxication, even though these subjects were heavy cigarette smokers.[15-19] There were parkinsonian clinical features with bradykinesia, rigidity, and clumsiness. It is of interest that the preliminary result of neuropsychological evaluation in our patients showed normal cognitive function except for visual perception, which is in contrast to the "manganese madness" reported in miners. This difference may be dose dependent since our patients had relatively mild disease.

Barbeau[20] emphasized that manganese neurotoxicity is best described as a low dopamine syndrome, with an early increase in dopamine turnover and more diffuse brain abnormalities than in Parkinson's disease. In addition to the neuropathological differences from Parkinson's disease, there are also some clinical points of distinction. Dyskinesias did not occur despite chronic treatment with high doses of levodopa.[21] Moreover, postsynaptic dopamine receptor supersensitivity, which is thought to occur in Parkinson's disease, could not be evoked in manganese-exposed rats.[22] Termination of manganese exposure tends to stabilize the clinical syndrome.

In conclusion, we share the opinion that the clinical picture of chronic manganese intoxication resembles Parkinson's disease. It may be compared as such with other infective, traumatic, or neurotoxic causes of parkinsonism such as encephalitis lethargica, pugilist's encephalopathy, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxication.[23]

We believe that the response to levodopa should be confirmed in a double-blind study, which we plan to undertake shortly.

**The authors are grateful to Mau-Sun Hua, PhD, for performing the neuropsychological tests on these patients.**

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# ATTACHMENT C-10

Phoon (1988)

LEADING ARTICLE

# MANGANESE EXPOSURE AND BIOLOGICAL INDICATORS

W H Phoon

SING MED J. 1988; 29:93–94

To evaluate an occupational health hazard often requires it to be quantified in order to assess the degree of the hazard to workers. A toxic chemical vapour in a workroom, for example, can be measured as to its level or concentration in the air, and the result compared to the recommended threshold limit value or TLV which is considered as a relatively "safe" level for the majority of workers. Such levels, however, only serve as guidelines in the control of potential health hazards.

Because of personal factors and susceptibility of workers, however, knowledge of the air level of a toxic chemical alone may not be sufficient to accurately evaluate a hazard. Biological indicators are useful in monitoring the health of individual workers and these are used in medical surveillance programmes. Such biological TLVs or exposure indices (BEIs) "represent warning levels of biological response to the chemical, or warning levels of the chemical or its metabolic product(s) in tissues, fluids or exhaled air of exposed workers" (1). Lead is a classical example of a substance for which blood concentrations have long been considered of critical value in determing "safe" and "unsafe" exposures.

In the case of manganese, however, the situation is less certain. Although manganese is an essential trace element in man, being a metalloprotein component of some enzymes like pyruvate decarboxylase, in excessive amounts it is a toxic metal which is known to be able to cause parkinsonism. Milder manifestations of poisoning include headache, restlessness, irritability and dysarthria (2,3). Cases of manganese poisoning have been reported among miners, and in workers in the production of alloys and dry cell batteries. A TLV of 5 mg/m³ air for an 8 hour-a-day exposure has been set (1). This was based on a few epidemiological studies which reported that poisoning cases had occurred at exposures much above this level and cases did not seem to occur below it (4). However, there have been other reports of adverse effects to the central nervous system in some workers exposed to air levels of only 2 to 5 mg/m³ (2).

**Department of Industrial Health**
**Ministry of Labour**
**5 Halifax Road**
**Singapore 0922**

Phoon Wai Hoong, MBBS (S), MSC (Occ Med) (Lon)

DIH (Eng), MFOM (RCP Lon), AM

Director

There is poor correlation between mean manganese-in-air levels and the degree of disorders (2). Various authors found no correlation between the level of manganese in the blood and that in the air. Most authors have found no direct relationship between blood and urine manganese levels and the occurrence or severity of poisoning (5). Studies of individuals with well developed signs and symptoms have revealed blood manganese levels within the normal range. In contrast, healthy miners may have increased blood levels (6). Individual susceptibility is thought to be a decisive factor in manganese toxicity (7).

However, Tanaka and Lieben found, on a group basis, that the urine level of manganese had some correlation with the average air concentration (8). A level of 50 μg Mn/l urine was proposed by the UK authority some years ago as a guideline to safe exposures (9).

Generally, the level of manganese in the blood, and especially in the urine, may be used to indicate the average level of exposure, on a group basis, but not on an individual basis (2,7). In individual workers, the blood and urine levels may be used to confirm exposure to manganese and possibly manganism. But no biological TLV or BEI can yet be proposed (2,5). The principle of biological monitoring for manganese exposure can only be recommended with reservation. Studies to date do not show a dose-response relationship for urine manganese and health disorders (7).

The paper on "Study of workers exposed to manganese dust in the dry cell battery manufacturing and manganese milling industries" in this journal reports that the air levels of manganese correlated significantly with those in the urine and blood of exposed workers, and that at the TLV of 5 mg/m³, the corresponding blood manganese concentration was about 30 μg/l and that in the urine was also about 30 μg/l. These are interesting findings and more such studies need to be done to confirm the relationship. If confirmed, such biological levels could be used, especially on a group basis, to complement the information derived from air monitoring. However, before a "biological TLV" can be proposed, especially for individual workers, it would be necessary to determine the levels of manganese in biological media (eg. blood, urine) at which adverse health effects are observed (2).

Further research also needs to be undertaken into the mechanisms of uptake and clearance of manganese from the respiratory system and gastrointestinal tract (2). It is thought that particle size plays a key role in this as it influences the deposition sites in the respiratory tract and the solubility rate (2,10). Only particles which are "respirable" (ie less than about 10 μ in size) can reach the alveoli. Larger particles are

cleared from the respiratory tract and eventually swallowed. In the gastrointestinal tract, only about 3% of the ingested manganese is absorbed (2). It is interesting to note that the paper by S L Gan et al in this journal reported that the manganese dust was "non-respirable", the particle size ranging from 12.53 to 55.73 $\mu$. This would suggest that absorption of the manganese by the workers was via the gastrointestinal tract.

Much as a biological TLV is desirable in the monitoring of workers, especially on an individual basis, more research needs to be done before one can be proposed. However it would still be useful to serially compare the group average blood and urine manganese levels as an indication of improving or deteriorating exposure levels. This would complement air monitoring results. The WHO recommends "repeated screening of subjective symptoms and thorough clinical examination" at regular intervals together with estimations of the level of manganese in blood and urine samples (6).

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# ATTACHMENT C-11

Nogawa et al. (1973)

# Mangan wo shu to suru funjin ni yoru taiki osen no kokyūki ni oyobosu eikyō ni kan-suru kenkyū (Dai-ippō) Chūgakusei no kokyūki e no eikyō [STUDIES OF THE EFFECTS ON THE RESPIRATORY ORGANS OF AIR POLLUTION CONSISTING OF DUSTS COMPOSED MAINLY OF MANGANESE. (FIRST REPORT) EFFECTS ON THE RESPIRATORY ORGANS OF JUNIOR HIGH SCHOOL STUDENTS]

K. Nogawa, E. Kobayashi, M. Sakamoto,
N. Fukushima, A. Ishizaki, N Makino,
S. Kagamimori, Y. Hiramatsu, S. Kawano,
T. Kato, K. Kanekawa, Shōzō

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SCITRAN (SCIENTIFIC TRANSLATION SERVICE)
SANTA BARBARA, CALIFORNIA

# STUDIES OF THE EFFECTS ON THE RESPIRATORY ORGANS OF AIR POLLUTION CONSISTING OF DUSTS COMPOSED MAINLY OF MANGANESE. (FIRST REPORT) EFFECTS ON THE RESPIRATORY ORGANS OF JUNIOR HIGH SCHOOL STUDENTS

Kōji Nogawa*, Etsuko Kobayashi*, Masako Sakamoto*, Noriaki Fukushima*, Arinobu Ishizaki*, Noriyoshi Makino**, Sadanobu Kagamimori**, Yoshitake Hiramaru**, Shunichi Kawano**, Takayuki Katō**, Katsuko Kanekawa***, and Shōzō (****)*****

## I. Introduction

In recent years, air pollution has progressed in all sections, and its effects on the human organism have become an important problem. Research is being carried out on the effects of various pollutants, particularly the sulfur oxides.

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* Kanazawa University Faculty of Medicine, School of Hygiene.
** Kanazawa University Faculty of Medicine, School of Public Health.
*** Kanazawa University, Medical Techniques Junior College, Nursing Department.
**** [illegible] in foreign text.
***** [illegible] Hospital.

There are few industrial plants in the city of Kanazawa, and the state of air pollution is relatively silent with respect to the measurement results for the various pollutants which are usually considered as presenting problems. Thus, almost no concern has been felt about air pollution here. However, surveys and studies conducted in 1967 and 1968 revealed that large amounts of manganese were contained in the dust emitted from an industrial plant chiefly producing ferromanganese, and that the vicinity surrounding this plant was polluted [1 - 3].

As for the effects of manganese dust on the human organism, there are a number of reports concerning occupational exposure [4 - 7]. However, there are very few reports concerning the effects on the human organism of regional environmental pollution by manganese dust. The writers carried out a survey of junior high school students for the purpose of determining whether air pollution by dust consisting chiefly of manganese has an influence on the human organism, particularly on the respiratory organs.

## II. Conditions in the Area Surveyed

As is shown in Figure 1, plant N, which produces chiefly ferromanganese, is located in the northeastern part of Kanazawa city. The N junior high school (hereinafter called the "polluted school"), which was the target of this survey, is located at a short distance away from it (about 100 meters). The S junior high school, which was selected as the control school in this survey (hereinafter called the "control school"), is located at a distance of about 7 km away from the N plant, from which it is separated by the downtown part of Kanazawa city.

A report of the Ishikawa Prefectural Research Institute for [illegible] and Environmental Pollution [1] is available concerning the state of air pollution in various areas of Kanazawa city and in the area around the plant. In the following, [illegible]


Figure 1. Outline of the area surveyed

x — area in vicinity of plant; · — areas other than those in vicinity of plant; 1 — Wind rose (1969); 2 — Japan Sea; 3 — Sai river; 4 — S junior high school; 5 — Hokuriku Line; 6 — Nishi-Kanazawa; 7 — M junior high school; 8 — Asano river; 9 — Higashi-Kanazawa; 10 — N plant; 11 — M junior high school

places in the vicinity of the plant, as is shown in Figure 1. They are established at distances of 200 - 400 m away from the plant. Besides these three points in the vicinity of the plant, there are four other measuring points within the city. At all of the measuring points, continuous measurements are made of the dust fall, the manganese contained in the dust fall, and the sulfur oxide concentration. The results for these are shown in Figure 2, both for the vicinity of the plant and for the other areas. The dust fall in the vicinity of the plant is somewhat greater than in the other areas, but there is no great difference. On the other hand, as for the manganese contained in the dust fall, the annual average for the four points located away from the vicinity of the plant is 8 kg/km$^2$/ month or less. On the other hand, the value for the vicinity of the plant is about 200 kg/km$^2$/month — amounting to more than 20 times the previous value. It is obvious that the manganese contents in the vicinity of the plant are remarkably great. In Kanazawa city there has been an observable tendency for the sulfur oxide concentrations to

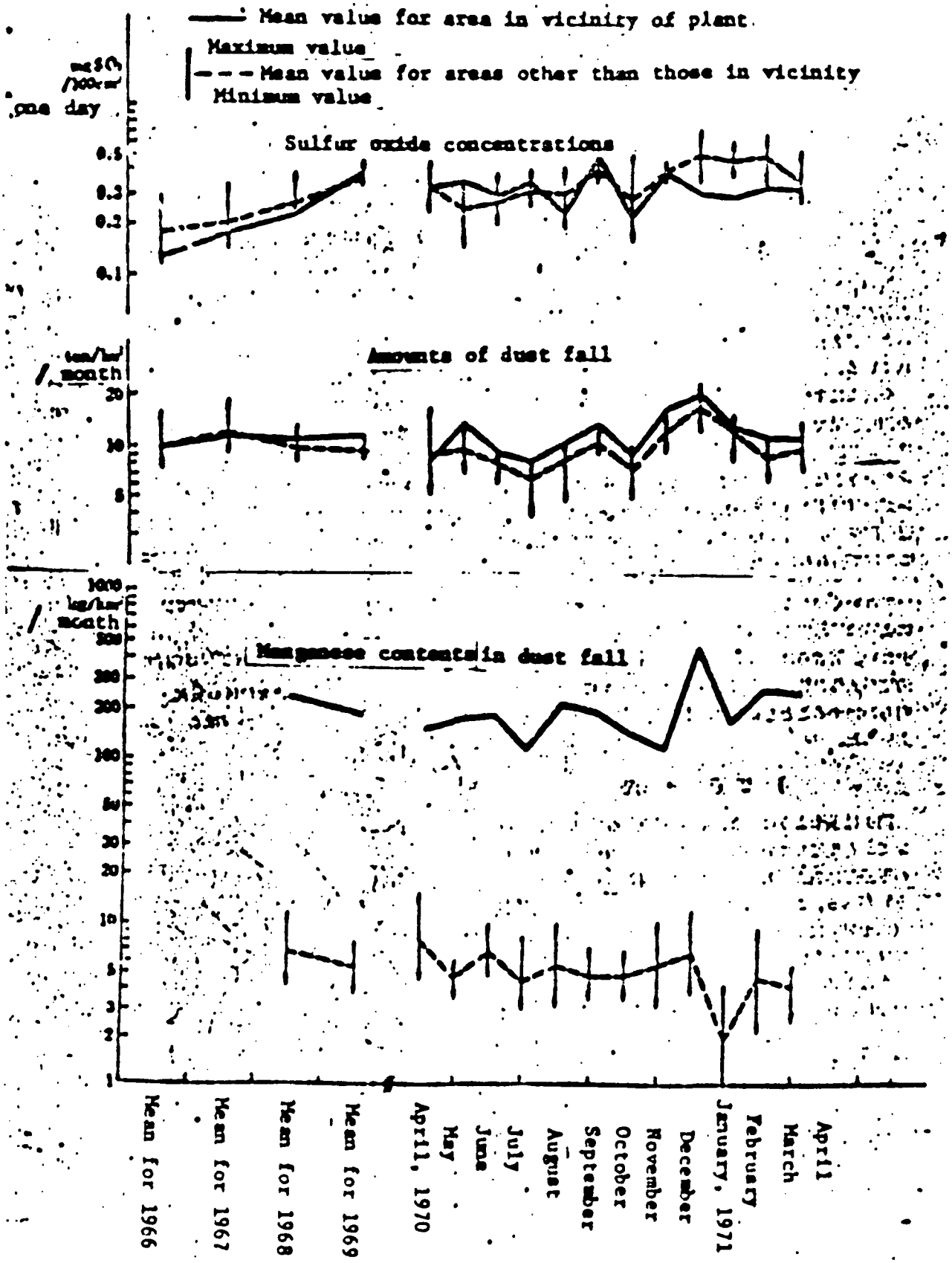
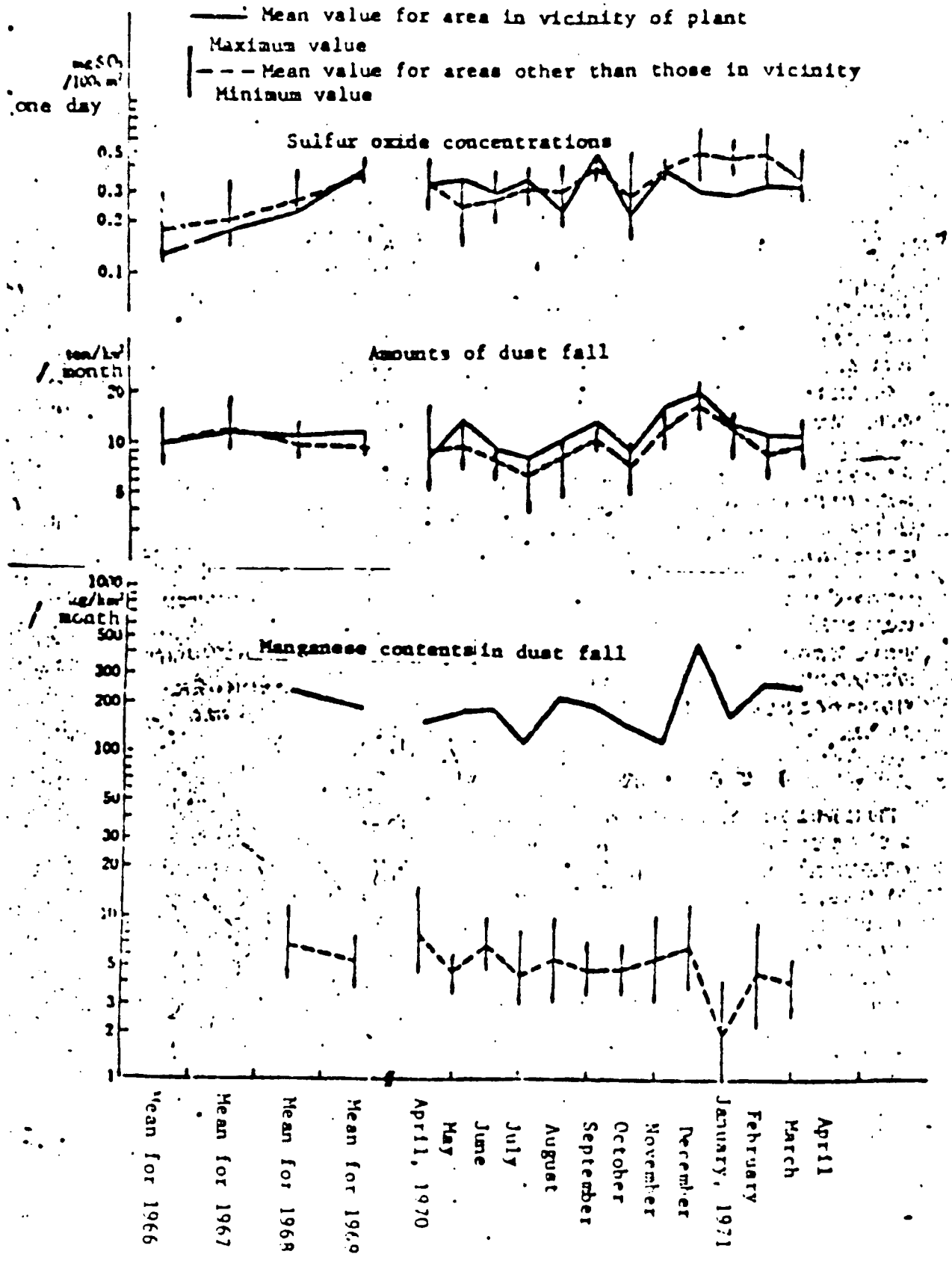



Figure 2. Sulfur oxide concentrations, amounts of dust fall, and manganese content in dust fall in Kanazawa city (surveyed by Ishikawa Prefectural Research Institute for Sanitation and Environmental Pollution)


Mean value for area in vicinity of plant
Maximum value
Mean value for areas other than those in vicinity
Minimum value
mg SO3 /100 cm² one day
Sulfur oxide concentrations
0.5
0.3
0.2
0.1
ton/km² month
Amounts of dust fall
20
10
8
1000 kg/km² month
Manganese contents in dust fall
500
300
200
100
50
30
20
10
5
3
2
1
Mean for 1966
Mean for 1967
Mean for 1968
Mean for 1969
April, 1970
May
June
July
August
September
October
November
December
January, 1971
February
March
April

increase year by year, but almost no difference can be observed between the vicinity of the plant and the other parts of the city.

When the range of the area affected by manganese pollution is studied in terms of the manganese content in cryptomeria leaves, the quantities are clearly great within a range of up to about 700 meters away from the plant [1]. When the manganese contained in the gutter dirt and in the cryptomeria leaves of this area was measured [2, 3], large quantities were found within a distance of 1,000 m away from the plant.

The N plant, which discharges the manganese dust, was built in 1938. At the time of the survey, it had four small-size electric furnaces of 1,000 - 3,500 kVA, as well as two large-size electric furnaces of 6,000 kVA and 7,500 kVA. It was producing mainly ferromanganese.

According to the 1970 measurement results of the Ishikawa Prefectural Research Institute for Sanitation and Environmental Pollution [1], the amounts of dust in the flue gas were 1.12 g/Nm$^3$ (maximum) and 0.02 g/Nm$^3$ (minimum). The manganese contents were 205 mg/Nm$^3$ (maximum) and 5 mg/Nm$^3$ (minimum).

## III. Population Surveyed

The population surveyed consisted of the junior high school students (all students belonging to the first, second, and third grades) of the polluted school (number of students enrolled: 1,258) and of the control school (number of students enrolled: 648). Approximately 9% of the students of the polluted school were living within a distance of 500 m away from the plant which was the source of pollution; approximately 24% of them were living at distances of 500 to 1000 m away from it; approximately 29% were living at distances of 1000 - 1500 m away; and approximately 38% were living at distances more than 1500 m away from it. Among the students of the control school, those living closest to the N plant were living at a distance of about 5 km away from it.

## IV. Survey Methods

### 1. Questionnaire Survey

Questionnaires of the type to be filled out by the informants were prepared. They contained the questions in the BMRC questionnaire [8] concerning subjective abnormalities in the eyes and throat, to which items concerning the state of health of other family members were added. The questionnaires were distributed to the students in advance, and were recovered on the day of the examination. The entries for each of the items were confirmed by interviews during the examinations.

### 2. Tests of Ventilation Functions

Three Tatebe recording Vitalor Meters were used, after it had been confirmed in advance that all three of them had identical performance properties. The forced expiratory volume, the one-second capacity, the one-second ratio, and the maximum expiratory flow were measured at both the polluted school and the control school by the identical methods, and by the same inspectors.

### 3. Survey Period

At the polluted school, the period was a five-day period beginning on July 13, 1970. At the control school, it was a three-day period beginning on July 22.

### 4. Percentages Surveyed and Examined

The rate of recovery of the questionnaire in the questionnaire survey was 98.2% of the number enrolled in the polluted school, and 98.8% in the control school.

The percentages who received tests of their ventilation functions were 97.1% in the polluted school, and 97.8% in the control school (Table 1).

TABLE 1. POPULATION SURVEYED AND NUMBERS AND PERCENTAGES OF THOSE SURVEYED AND EXAMINED

| | Categories | | Number enrolled | Questionnaire survey | | Tests of ventilation functions | |
|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % |
| Polluted school | Total (persons) | | 1,258 | 1,235 | 98.2 | 1,222 | 97.1 |
| | First grade: | males | 194 | 193 | 99.5 | 192 | 99.0 |
| | | females | 188 | 185 | 98.4 | 180 | 95.7 |
| | Second grade: | males | 207 | 203 | 98.1 | 202 | 97.6 |
| | | females | 193 | 192 | 99.5 | 191 | 99.0 |
| | Third grade: | males | 229 | 221 | 96.5 | 217 | 94.8 |
| | | females | 247 | 241 | 97.6 | 240 | 97.2 |
| Control school | Total | | 648 | 640 | 98.8 | 634 | 97.8 |
| | First grade: | males | 93 | 92 | 93.9 | 92 | 98.9 |
| | | females | 121 | 120 | 99.2 | 118 | 97.5 |
| | Second grade: | males | 114 | 110 | 96.5 | 110 | 96.5 |
| | | females | 91 | 93 | 93.9 | 93 | 98.9 |
| | Third grade: | males | 109 | 100 | 100.0 | 109 | 100.0 |
| | | females | 117 | 116 | 99.1 | 112 | 95.7 |

## V. Survey Results

### 1. Questionnaire Survey Results

#### 1) Comparison of polluted school and control school

Table 2 is a comparison of the males and females for their subjective symptoms, their past histories, their present symptoms, and the state of health of their families.

According to this, for both males and females the prevalences in the students of the polluted school are higher than in the control

TABLE 2. COMPARISON OF SUBJECTIVE SYMPTOMS, PAST HISTORY, PRESENT HISTORY, AND FAMILY HISTORY FOR THE TWO SCHOOLS

| Items | | Males | | | Females | | | Total, both M and F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Polluted school | Control school | t test | Polluted school | Control school | t test | Polluted school | Control school | t test |
| | | Persons (%) | | | Persons (%) | | | Persons (%) | | |
| Total | | 617 (100.0) | 311 (100.0) | | 618 (100.0) | 329 (100.0) | | 1235 (100.0) | 640 (100.0) | |
| Cough | Always in winter on arising (at least 5 days a week) | 4 (0.6) | 1 (0.3) | | 9 (1.5) | 8 (2.4) | | 13 (1.1) | 9 (1.4) | |
| | Ditto in summer | 0 — | 1 (0.3) | | 2 (0.3) | 3 (0.9) | | 2 (0.2) | 4 (0.6) | |
| | During day and at night in winter (at least 6 times a day) | 14 (2.3) | 4 (1.3) | | 21 (3.4) | 11 (3.3) | | 35 (2.8) | 15 (2.3) | |
| | Ditto in summer | 2 (0.3) | 1 (0.3) | | 5 (0.8) | 0 — | | 7 (0.6) | 1 (0.2) | |
| | Every day about 3 months a year | 2 (0.3) | 0 — | | 7 (1.1) | 4 (1.2) | | 9 (0.7) | 4 (0.6) | |
| Sputum | Always in winter on arising (at least 5 days a week) | 25 (4.1) | 4 (1.3) | * | 20 (3.2) | 8 (2.4) | | 45 (3.6) | 12 (1.9) | * |
| | Ditto in summer | 14 (2.3) | 5 (1.6) | | 9 (1.5) | 4 (1.2) | | 23 (1.9) | 9 (1.4) | |
| | During day and at night in winter (at least twice a day) | 32 (5.2) | 10 (3.2) | | 22 (3.7) | 11 (3.3) | | 54 (4.3) | 21 (3.3) | |
| | Ditto in summer | 25 (4.1) | 7 (2.3) | | 18 (2.9) | 4 (1.2) | | 43 (3.5) | 11 (1.7) | * |
| | Every day about 3 months a year | 12 (1.9) | 2 (0.6) | | 9 (1.5) | 1 (0.3) | | 21 (1.7) | 3 (0.5) | |
| Coughing and sputum continuing for at least 3 wks during past 3 yrs | | 7 (1.1) | 2 (0.6) | | 7 (1.1) | 2 (0.6) | | 14 (1.1) | 4 (0.6) | |
| Blood mixed with sputum | | 8 (1.3) | 2 (0.6) | | 14 (2.3) | 4 (1.2) | | 22 (1.8) | 6 (0.9) | |
| Wheezing | Yes | 99 (16.0) | 44 (14.1) | | 134(21.7) | 59 (17.9) | | 233(18.9) | 103(16.1) | ** |
| | Yes (after catching a cold) | 73 (11.8) | 37 (11.9) | | 144 (20.9) | 55 (16.7) | | 217(17.6) | 92 (14.4) | ** |
| | Yes (not during a cold) | 18 (2.9) | 8 (2.6) | | 16 (2.6) | 3 (0.9) | | 34 (2.8) | 11 (1.7) | |
| | Almost every day or every night | 3 (0.5) | 0 — | | 8 (1.3) | 1 (0.3) | | 11 (0.9) | 1 (0.2) | |

The [illegible] following [illegible]

TABLE 2. (continued)

| Items | Male | | | Female | | | Total, both M and F | | |
|---|---|---|---|---|---|---|---|---|---|
| | Polluted school | Control school | Test | Polluted school | Control school | Test | Polluted school | Control school | Test |
| Total | Persons (%) | | | Persons (%) | | | Persons (%) | | |
| Past history of attacks of wheezing and shortness of breath | 16 (2.6) | 8 (2.6) | | 20 (3.2) | 6 (1.8) | | 36 (2.9) | 14 (2.2) | |
| Current attacks of wheezing and shortness of breath | 11 (1.8) | 2 (0.6) | | 8 (1.3) | 4 (1.2) | | 19 (1.5) | 6 (0.9) | |
| Clogged nose, nose colds: Frequently in winter | 226(36.3) | 157(50.5) | ** | 400(64.7) | 188(57.1) | ** | 726(50.6) | 345(53.9) | ** |
| Clogged nose, nose colds: Frequently in summer | 85 (13.4) | 51 (16.0) | * | 74 (12.0) | 21 (6.4) | ** | 160(12.7) | 72 (11.1) | ** |
| Clogged nose, nose colds: Every day about 3 months in the year | 35 (5.7) | 11 (3.5) | | 30 (4.9) | 10 (3.0) | | 65 (4.5) | 21 (3.3) | |
| Had respiratory illness during past 3 yrs (for one week or longer) | 17 (2.8) | 4 (1.3) | | 14 (2.3) | 6 (1.8) | | 31 (2.5) | 10 (1.6) | |
| Increase in sputum in the above-mentioned illness | 11 (1.8) | 3 (1.0) | | 8 (1.3) | 4 (1.2) | | 19 (1.5) | 7 (1.1) | |
| Eye symptoms: During the winter | 41 (6.6) | 23 (7.4) | | 45 (7.3) | 23 (7.6) | | 86 (7.0) | 46 (7.5) | |
| Eye symptoms: During the summer | 63 (10.2) | 22 (7.1) | | 68 (11.0) | 25 (7.6) | ** | 131(10.6) | 47 (7.3) | * |
| Eye symptoms: Every day about 3 months in the year | 7 (1.1) | 3 (1.0) | | 5 (0.8) | 1 (0.3) | | 12 (1.0) | 4 (0.6) | |
| Irritant symptoms (swelling, soreness): During the winter | 199(32.3) | 80 (25.7) | * | 313(50.6) | 117(35.2) | * | 512(41.5) | 222(34.7) | ** |
| Irritant symptoms (swelling, soreness): During the winter (after catching a cold) | 195(31.6) | 72 (23.2) | ** | 303(49.1) | 110(33.4) | * | 500(40.5) | 212(33.1) | ** |
| Irritant symptoms (swelling, soreness): During the winter (not during a cold) | 26 (4.2) | 8 (2.6) | | 29 (4.7) | 6 (1.8) | * | 55 (4.5) | 14 (2.2) | * |
| Irritant symptoms (swelling, soreness): During the summer | 42 (6.8) | 9 (2.9) | * | 53 (8.6) | 21 (6.4) | | 95 (7.7) | 30 (4.7) | * |
| Irritant symptoms (swelling, soreness): During the summer (after catching a cold) | 36 (5.8) | 7 (2.3) | * | 47 (7.6) | 20 (6.1) | | 83 (6.7) | 27 (4.2) | * |
| Irritant symptoms (swelling, soreness): During the summer (not during a cold) | 14 (2.3) | 3 (1.0) | | 10 (1.6) | 1 (0.3) | | 24 (1.9) | 4 (0.6) | * |

TABLE 2. (concluded)

| Items | | Male | | | Female | | | Total, both M and F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Polluted school | Control school | Test | Polluted school | Control school | Test | Polluted school | Control school | Test |
| Total | | Persons (%) | | | Persons (%) | | | Persons (%) | | |
| | Every day about 3 months in the year | 2 (0.3) | 0 — | | 9 (1.5) | 5 (1.5) | | 11 (0.9) | 5 (0.8) | |
| Pointed out by doctor | Pneumonia | 43 (7.0) | 14 (4.5) | | 57 (9.5) | 19 (5.8) | | 100 (8.1) | 33 (5.1 | * |
| | Pleurisy | 2 (0.3) | 0 — | | 0 — | 0 — | | 2 (0.2) | 0 — | |
| | Tuberculosis | 6 (1.0) | 1 (0.3) | | 2 (0.3) | 1 (0.3) | | 8 (0.6) | 2 (0.3) | |
| Past, current history | Bronchitis | 43 (7.0) | 24 (7.7) | | 46 (7.4) | 33 (10.0) | | 89 (7.2) | 57 (8.9) | |
| | Bronchial asthma | 23 (3.7) | 10 (3.2) | | 17 (2.8) | 5 (1.5) | | 40 (3.2) | 15 (2.3) | |
| | Sinus empyema | 60 (9.7) | 14 (4.5) | ** | 22 (3.2) | 11 (3.3) | | 82 (7.4) | 25 (3.9) | ** |
| | Heart disease | 5 (0.8) | 3 (1.0) | | 6 (1.0) | 4 (1.2) | | 11 (0.9) | 7 (1.1) | |
| | Conjunctivitis | 51 (8.3) | 24 (7.7) | | 57 (9.2) | 29 (8.8) | | 108 (8.7) | 53 (8.3) | |
| Diseases of family members living in the same house | Chronic bronchitis | 11 (1.8) | 5 (1.6) | | 11 (1.8) | 2 (0.6) | | 22 (1.8) | 7 (1.1) | |
| | Pulmonary pneumonia | 7 (1.1) | 0 — | | 7 (1.1) | 1 (0.3) | | 14 (1.1) | 1 (0.2) | |
| | Asthma | 21 (3.5) | 9 (2.9) | | 26 (4.2) | 10 (3.0) | | 50 (4.0) | 19 (3.0) | |
| | Pneumonia | 5 (0.8) | 1 (0.3) | | 8 (1.3) | 2 (0.6) | | 13 (1.1) | 3 (0.5) | |
| | Persons prone to coughing and sputum | 50 (8.1) | 13 (4.2) | * | 42 (6.8) | 18 (5.8) | | 92 (7.4) | 22 (3.6) | * |

sug — $0.05 \leq p < 0.1$; * — $0.01 \leq p < 0.05$;
** — $p < 0.01$.

school, with the exception of some of the items such as "cough". As for the individual items, in the males, the percentages for the following items are significantly higher in the students of the polluted school: "sputum always in the winter on arising", "clogged nose, nose colds frequently in the summer", "throat symptoms", "past history of sinus empyema", "some members of the family living in the same house have cough and sputum lasting for longer than two months". In the females, the percentages for all [illegible] items are significantly

higher in the students of the polluted school: "clogged nose, nose colds", "throat symptoms in winter". For the other items, when the percentages for "eye symptoms" and "past history of pneumonia" are added together for both males and females, the percentages are significantly higher in the polluted school. However, there was not a single student, either in the polluted school or in the control school, corresponding to the definition of chronic bronchitis on the basis of the BMRC instruction manual [9].

2) Comparisons of different grades in the polluted school

The different grades in the polluted school were compared for those of the survey items in which significant differences could be seen between both schools. The first-grade students had been coming to the polluted school for a period of about three months. Unlike the second and third graders, who had been coming to the school for longer than one year, they were believed to have been exposed to less effects of the pollution in the school. For this reason, the students were divided into two groups: the first graders, and the second and third graders, and the percentages of the subjective symptoms were compared. No differences between the grades could be seen, except for the fact that "throat symptoms during the winter" appeared with a significantly higher percentage in the second and third graders in comparison with the first graders.

3) Comparisons by place of residence

A study was made according to the place of residence, the residences being classified according to the distance away from the N plant, the pollution source, for those items among the subjective symptoms, the past history, the current history, and the state of health of the family members for which there was a significantly higher percentage among students of the polluted school. The results are shown in Figure 3. There was a tendency for those students whose residences were close to the source of pollution to have a higher

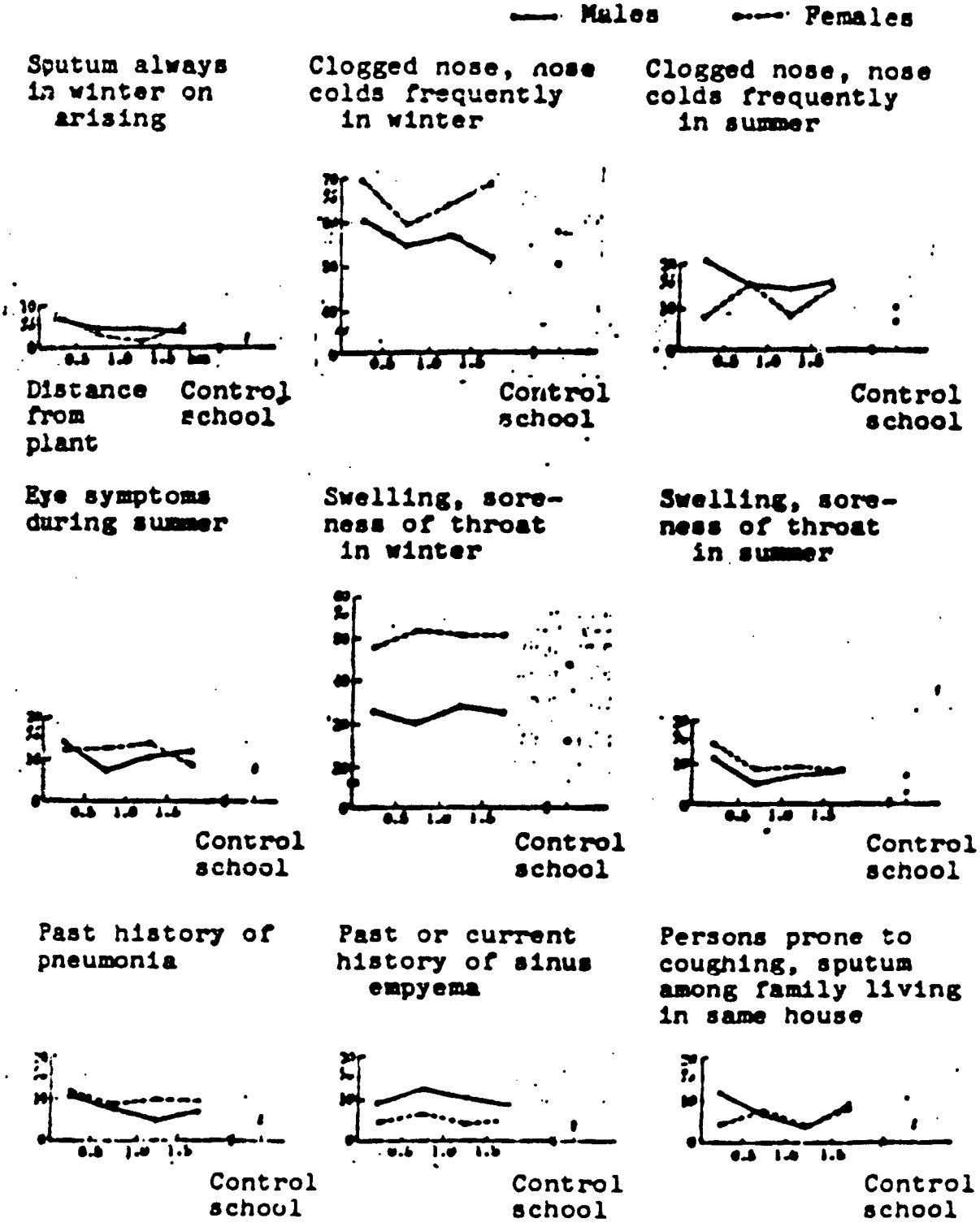



Figure 3. Subjective symptoms arranged by place of residence (polluted school)

percentage for two items: "throat swelling and soreness in summer and "past history of pneumonia".

## 2. Results of Ventilation Function Tests

### 1) Comparison of polluted school and control school

The mean values of the vital capacity, the one-second capacity, the one-second ratio, and the maximum expiratory flow are shown in Table 3 for the different schools, for the different grades, and for the males and females. Since these numerical values indicating the ventilation functions are influenced by the stature, the mean values of the body heights and body weights for the students of both schools were also indicated. Significant differences were seen in the body heights. That is, the second-grade males had a greater body height in the control school, but the first-grade females had a greater body height in the polluted school. However, these differences are not pronounced, and it was therefore assumed that there would be no need to consider that particular differences in stature existed between the groups of students of both schools. Thus, it was decided to compare the mean values for their ventilation functions without any modifications.

In the mean values for the vital capacity, the students of the polluted school had lower values than the students of the control school. This applied to both males and females, and to all three grades. However, significant differences were seen only in the first and second grade males and in the first grade females. In the mean values for the one-second capacity, the students of the polluted school had significantly lower values. This applied to both males and females and to all grades. As for the mean values of the one-second ratio, the students in the polluted school displayed results ranging from 0.7% (first grade males) to 5.3% (third grade females) lower than those in the control school. These differences were significant in all except the first grade males. The mean values of the maximum expiratory flow increased with each grade, but in

TABLE 3. RESULTS OF TESTS OF PULMONARY VENTILATION FUNCTIONS

| Items | | Males | | Females | |
|---|---|---|---|---|---|
| | | Polluted school | Control school | Polluted school | Control school |
| | | M ± S.E. | M ± S.E. | M ± S.E. | M ± S.E. |
| Vital capacity (l) | 1st grade | 2.41±0.031* | 2.58±0.000 | 2.16±0.030** | 2.37±0.033 |
| | 2nd grade | 2.83±0.037* | 2.86±0.037 | 2.41±0.027 | 2.49±0.035 |
| | 3rd grade | 3.21±0.011 | 3.33±0.039 | 2.56±0.021 | 2.58±0.016 |
| 1-sec capacity (l) | 1st grade | 2.03±0.029* | 2.21±0.018 | 1.86±0.026** | 2.04±0.031 |
| | 2nd grade | 2.36±0.031** | 2.67±0.030 | 2.05±0.027** | 2.18±0.033 |
| | 3rd grade | 2.70±0.026** | 2.83±0.018 | 2.14±0.023** | 2.23±0.031 |
| 1-sec ratio (%) | 1st grade | 83.4±0.56 | 86.1±0.80 | 86.7±0.67** | 86.9±0.63 |
| | 2nd grade | 83.1±0.73** | 87.4±0.53 | 81.5±0.75** | 87.9±0.78 |
| | 3rd grade | 83.3±0.63** | 87.1±0.73 | 83.6±0.67** | 83.1±0.79 |
| Maximum expiratory flow (l/min) | 1st grade | 199±3.3* | 222±7.1 | 136±2.6** | 181±3.1 |
| | 2nd grade | 215±4.5** | 223±6.3 | 160±3.3* | 183±3.3 |
| | 3rd grade | 231±3.0** | 273±6.9 | 176±2.2** | 203±4.9 |
| Body height (cm) | 1st grade | 118.1±0.51 | 117.9±0.78 | 138.1±0.46** | 118.0±0.51 |
| | 2nd grade | 131.1±0.55* | 130.9±0.72 | 132.4±0.41 | 133.5±0.53 |
| | 3rd grade | 150.8±0.51 | 150.8±0.73 | 135.1±0.53 | 131.8±0.49 |
| Body weight (kg) | 1st grade | 20.6±0.56 | 20.6±0.74 | 20.0±0.47 | 20.8±0.63 |
| | 2nd grade | 41.7±0.53 | 43.8±0.75 | 41.9±0.42 | 43.1±0.61 |
| | 3rd grade | 50.8±0.37 | 52.6±0.85 | 49.1±0.43 | 49.5±0.61 |

Difference between polluted school and control school: *0.01 ≤ P < 0.05; **P < 0.01

TABLE ... RESULTS OF TESTS OF PULMONARY VENTILATION FUNCTIONS

| Items | | Males | | Females | |
|---|---|---|---|---|---|
| | | Polluted school | Control school | Polluted school | Control school |
| | | M ± S.E. | M ± S.E. | M ± S.E. | M ± S.E. |
| ... | 1st grade | 2.41±0.071* | 2.58±0.060 | 2.16±0.020** | 2.27±0.033 |
| ... | 2nd grade | 2.83±0.019** | 3.06±0.037 | 2.41±0.027 | 2.49±0.035 |
| ... | 3rd grade | 3.2...±0.011 | 3.33±0.059 | 2.56±0.021 | 2.58±0.014 |
| ... | 1st grade | 2.03±0.079* | 2.21±0.016 | 1.86±0.030** | 2.01±0.031 |
| ... | 2nd grade | 2.36±0.011** | 2.67±0.030 | 2.03±0.027** | 2.18±0.033 |
| ... | 3rd grade | 2.70±0.035** | 2.63±0.018 | 2.11±0.023** | 2.29±0.031 |
| ... | 1st grade | 85.4±0.56 | 86.1±0.69 | 86.7±0.67** | 89.9±0.63 |
| ... | 2nd grade | 83.1±0.70** | 87.4±0.53 | 81.5±0.75** | 87.9±0.78 |
| ... | 3rd grade | 83.5±0.67** | 87.1±0.73 | 83.8±0.67** | 89.1±0.79 |
| ... | 1st grade | 199±3.3* | 222±7.1 | 155±3.6** | 181±5.1 |
| ... | 2nd grade | 215±4.5** | 253±6.3 | 169±3.5* | 185±5.3 |
| ... | 3rd grade | 231±5.0** | 275±6.9 | 176±3.8** | 203±4.9 |
| ... | 1st grade | 119±0.51 | 137.9±0.78 | 150.1±0.46** | 148.0±0.51 |
| ... | 2nd grade | 151.1±0.55* | 156.2±0.72 | 152.4±0.41 | 153.5±0.55 |
| ... | 3rd grade | 160.8±0.51 | 160.8±0.75 | 155.1±0.35 | 151.8±0.43 |
| ... | 1st grade | 35.4±0.56 | 39.6±0.71 | 40.0±0.17** | 40.8±0.63 |
| ... | 2nd grade | 41.7±0.53 | 45.8±0.75 | 41.9±0.42 | 45.1±0.61 |
| ... | 3rd grade | 46.0±0.57 | 50.6±0.80 | 44.1±0.43 | 43.9±0.61 |

...ifference between polluted school and control school: *0.01 ≤ P < 0.05;
**P < 0.01

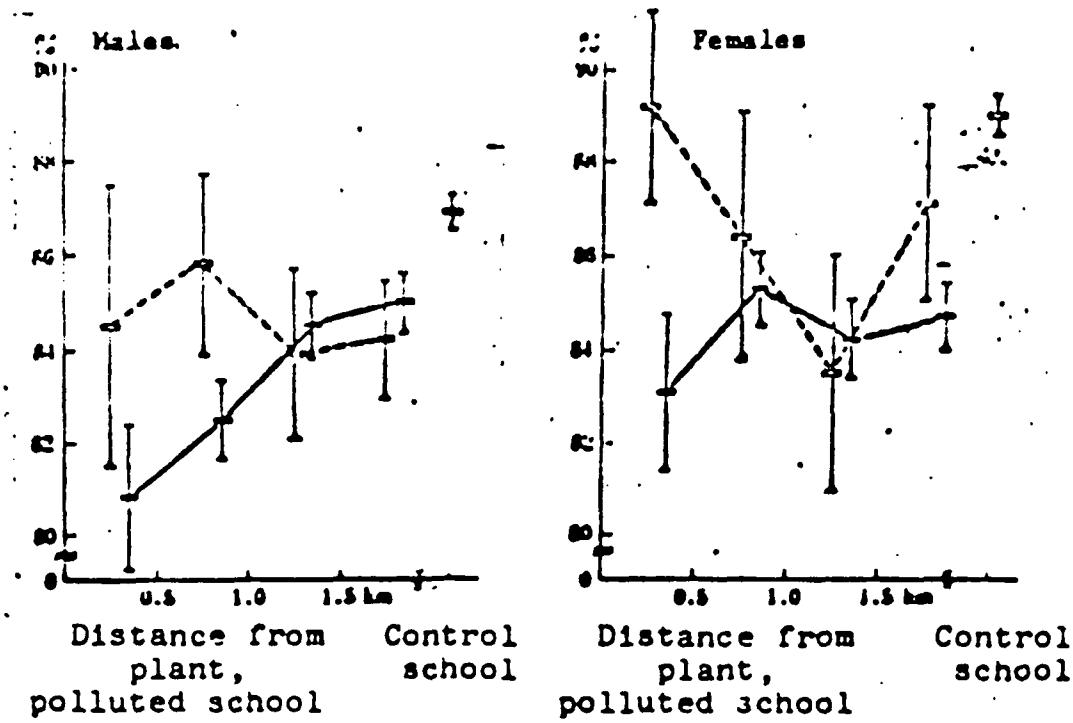


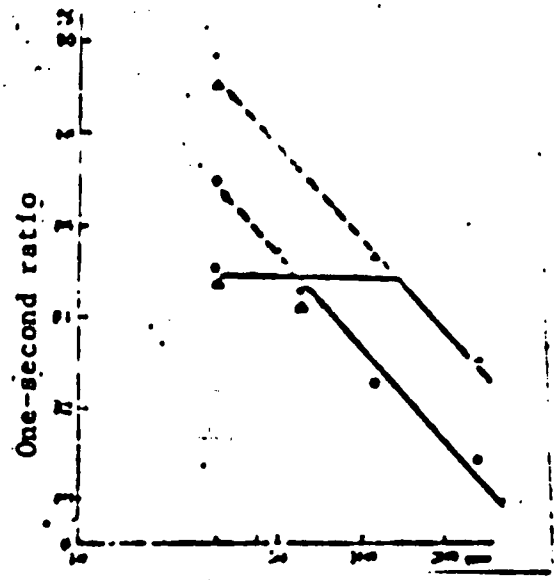

Figure 4. One-second ratio classified by places or residence and by length of residence (all grades): ▬▪▬ — residing for less than three years; ▬▬ — residing for more than three years; M. ± S.E.

Among the females, the tendencies were not as clear as among the males. However, those students who had been living near the plant for longer than three years displayed a lower one-second ratio, just as among the males. As for the period of residence, those who had been living there for less than three years had one-second ratios which were somewhat higher than those of the students who had been living there for more than three years.

### 4) Relationship between one-second ratio and manganese content in cryptomeria leaves and in gutter dirt

Reports by Suzuki et al. [2, 3] are available concerning the [illegible] environmental pollution of the plant vicinity by manganese. [illegible] between the manganese concentration in the crypto- [illegible] gutter dirt and the [illegible]

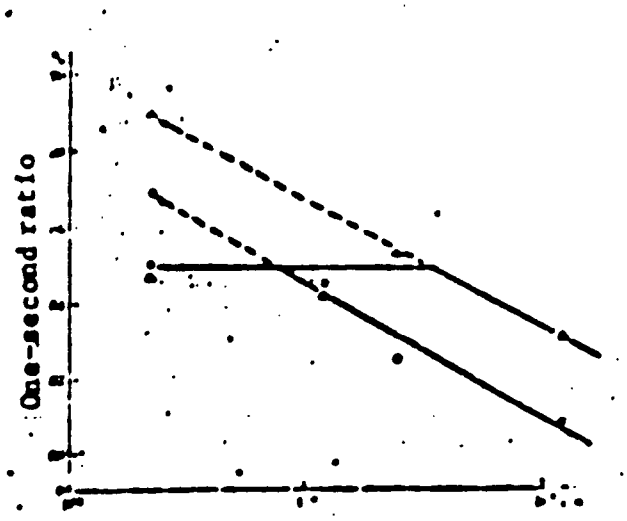
in this survey are shown in Figures 5 and 6, in relation to the distance away from the plant.




Mn content, cryptomeria leaves (from [2])

Figure 5. Relationship between manganese content in cryptomeria leaves and one-second ratio (those residing more than three years:

● — polluted school, males; ▲ — polluted school, females; ○ — control school, males; △ — control school; females




Mn content, gutter dirt (from [3])

Figure 6. Relationship between manganese content in gutter dirt and one-second ratio (those residing for more than three years):

● — polluted school, males; ▲ — polluted school, females; ○ — control school males; △ — control school, females

The one-second ratios were found to be lower in those places where there were high manganese concentrations in the cryptomeria leaves and in the gutter dirt. The measured values for the control school were located more or less on a line following in direct projection from the manganese concentration line and the line indicating the tendency of the one-second ratio to drop for those living near the plant.

comparisons between the two schools, the students of the polluted school also had significantly lower values. This applied both to males and females and to all the grades.

2) Comparisons of different grades

Among tests of the ventilation functions, the one-second ratio is regarded as being relatively unaffected by the stature. In this survey, an analysis of variance was made for the students of the control school with reference to presence or absence of differences between the grades in the one-second ratio. The results are as shown in Table 4. It is clear that one cannot say that there are differences between the different grades, either for the males or for the females. On the other hand, when the same analysis of variance was made for the students of the polluted school, the differences between the grades was found to be significant, both for the males and for the females. The results indicated that there was a great decline in the one-second ratio in the students of the upper grades.

3) Comparisons by places of residence and length of residence

Differences for length of residence and for place of residence were studied for the one-second ratio of the male and female students of the polluted school. The results are given in Figure 4. The students were divided into two groups according to their length of residence: those who had lived for less than three years at their present address; and those who had lived there for longer than three years. For the place of residence, the students were classified according to their distance away from the plant which was the pollution source.

Among the males, those students who had lived for longer than three years at a distance of less than 0.5 km from the plant had a one-second ratio averaging 80.8%. Those who had lived for the same period at a distance of 0.5 - 1.0 km had an average of 82.5%. Those who had lived for the same period at a distance of 1.0 - 1.5 km had

TABLE 4. ANALYSIS OF VARIANCE OF ONE-SECOND RATIO FOR DIFFERENT GRADES

Polluted school

| | Factors | Fluctuations (sum of squares) | Degree of freedom | Unbiased estimate of population variance (mean square) | Mean square ratio |
|---|---|---|---|---|---|
| Males | Between grades | 627.41 | 2 | 313.71 | 3.82* |
| | Within grades | 49914.85 | 608 | 82.10 | |
| | Total fluctuation | 50512.26 | 610 | | |
| Females | Between grades | 942.30 | 2 | 471.15 | 4.70** |
| | Within grades | 60987.18 | 608 | 100.31 | |
| | Total fluctuation | 61929.48 | 610 | | |
| Control school | | | | | |
| Males | Between grades | 91.98 | 2 | 45.99 | 1.10 |
| | Within grades | 12932.07 | 308 | 41.99 | |
| | Total fluctuation | 13024.05 | 310 | | |
| Females | Between grades | 201.50 | 2 | 100.75 | 1.76 |
| | Within grades | 18333.64 | 320 | 57.29 | |
| | Total fluctuation | 18535.14 | 322 | | |

*$0.01 \leq P < 0.05$.

**$P < 0.01$.

an average of 84.5%, and those who had lived for the same period at a distance of more than 1.5 km had an average of 85.0%. Thus, the one-second ratio was lower, the closer the students lived to the plant. However, for those who had lived at their present addresses for less than three years, no differences by place of residence could be observed. They displayed approximately the same one-second ratio as those who had been living for longer than three years at a considerable distance away from the plant.

## VI. Considerations

In Kanazawa city and its environs there are few industrial plants and no thermal power stations. The state of atmospheric pollution was believed to be relatively light, and little attention was paid to problems of air pollution. However, local manganese pollution in the vicinity of a plant producing ferromanganese was pointed out by Suzuki et al. [2, 3], and attention came to be focused on the effects of pollution of this type on the human organism.

Continuous measurements of the dust fall, the sulfur oxide concentration, and the suspended dust had been made by the Ishikawa Prefectural Research Institute for Sanitation and Environmental Pollution as general indexes of air pollution in Kanazawa city. Therefore, these results were divided up into the places in the vicinity of the plant in question and the other points, and the data were analyzed. However, pronounced differences could not be observed for the dust fall or for the sulfur oxide concentration. That is, the amount of dust fall in the area surrounding the plant was approximately the same as that at the point displaying the maximum value among the other four points in the city. The amount was also far lower than the dust fall of 30 $t/km^2/month$, at which the peak-flow values of the schoolchildren are said to decline [10]. As for the sulfur oxide concentration, the value in the area surrounding this plant was even lower than the values at the other four points in the city. However, the manganese content in the dust fall alone had a much higher value in the vicinity of this plant; and the concentration was more than 20 times the mean values for the other four points in the city. The writers took samples of suspended dust inside and outside the plant premises and analyzed them for their heavy metals content (Table 5). As is clear from the results of this analysis, the dust discharged from this plant has a high manganese content. As for the manganese content in the suspended dust, it was 15 - 30% inside the plant premises, and 1.8% outside the plant premises. The manganese concentration in the flue gas amounts to 7.6 - 25.1% [1]. The manganese is followed by iron (Fe), which is also present in considerable quantities, but almost no other metals are contained.

TABLE 5. ATMOSPHERIC SUSPENDED DUST AND CONCENTRATIONS OF HEAVY METALS (BY HIGH VOLUME AIR SAMPLER)

| Items / Place of measurement | Suspended dust | | Mn | | Fe | | Pb | | Cu | | Cd | | Zn | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc., $mg/m^3$ | % | Conc., $\mu g/m^3$ | % | Conc., $\mu g/m^3$ | % | Conc., $\mu g/m^3$ | % | Conc., $\mu g/m^3$ | % | Conc., $\mu g/m^3$ | % | Conc., $\mu g/m^3$ | % |
| Inside the plant: Blending work room | 1.625 | 100 | 310 | 20.9 | 104 | 6.4 | 0.33 | 0.02 | 0.73 | 0.05 | 0.022 | – | 7 | 0.43 |
| Sintering | 5.255 | 100 | 1570 | 29.9 | 335 | 6.8 | 0.67 | 0.01 | 1.82 | 0.03 | 0.105 | – | 14 | 0.27 |
| By small-size electric furnaces | 5.115 | 100 | 740 | 14.5 | 524 | 10.3 | 3.84 | 0.07 | 15.1 | 0.30 | 0.069 | – | | |
| By large-size electric furnaces | 5.565 | 100 | 860 | 15.4 | 696 | 12.5 | 2.50 | 0.05 | 6.4 | 0.12 | 0.035 | – | 24 | 0.43 |
| 100 m away from plant | 0.299 | 100 | 4.04 | 1.8 | 1.27 | 0.6 | 0.18 | 0.08 | 0.14 | 0.06 | 0.003 | – | | |

When measurements were made of the smoke after it had been followed downwind, the maximum values of the suspended dust and the manganese concentration are reported to have been 1.7 $mg/m^3$ and 260 $\mu g/m^3$, respectively [1]. If one supposes, from Table 5, that the iron concentration in the atmosphere in the area surrounding the plant is about one-third of the manganese concentration, the iron concentration will be estimated at 87 $\mu g/m^3$. If these values are compared with the maximum values in the seven main cities for the year 1968 [11], the values for the suspended dust are twice the maximum values, the manganese concentration has a value 74 times the maximum values, and the iron concentration is three times the maximum values. As for the amount of suspended dust in the atmosphere and the manganese concentration at a point about 300 meters away from the plant, five-day average values of 160 $\mu g/m^3$ and 0.7 $\mu g/m^3$, respectively, have been reported [1]. If the iron concentration is estimated in the manner described above, it will have an assumed value of 2.2 $\mu g/m^3$. When these values are compared with the maximum and minimum mean values in the main cities, the suspended dust has a concentration of 0.9 - 0.4 times, the manganese has a concentration of 44.7 - 9.3 times, and the iron has a concentration of 0.6 - 0.1 times the mean values. Studies were similarly made for lead (Pb) and copper (Cu). Their amounts were definitely not especially great. In this way, in the vicinity of this plant, only the suspended dust and the manganese concentration, among the various heavy metals which were measured, were remarkably high.

On the other hand, there are many reports concerning the influence of air pollution on the respiratory functions. Toyama [12], Kuroda [13], Watanabe [14], and Prindle et al. [15] make comparisons of the respiratory functions between persons in air-polluted areas and in non-polluted areas. The amounts of the dust fall, the sulfur oxide concentrations, and the amounts of suspended dust in the non-polluted areas in these reports either differ little from or are even higher than the measured values in the area surrounding the plant in this survey.

Consequently, it is necessary to focus the attention squarely on manganese in the pollution of the area studied in this survey. In studies of occupation exposure, it is reported that the chief toxic effects of manganese are disturbances of the nervous system, and disturbances of the respiratory system, the chief disturbance being "manganese pneumonia" [16, 17]. When considering manganese in air pollution, which has a much lower concentration than in occupational exposure, one must first of all take into consideration the effects on the respiratory system.

The population surveyed in this survey consisted of junior high school students. Junior high school students were chosen here for the following reasons. First of all, there are no effects of smoking in junior high school students. There are no occupational effects. They are capable of understanding the respiratory function tests and the questionnaires. Furthermore, they live in a definite area, and it is possible to secure a high percentage of examination. In addition to these general advantages, in this case a junior high school was located close to the plant. Tatebe recording Vitalor Meters were used for measuring the pulmonary functions in this case for the following reasons. First of all, the equipment is simple to use and can be used for many surveys. Furthermore, it has been reported that the Vitalor is useful in the surveys of Harden [18] and of Yamazaki [19]. It has been reported that surveys of the respiratory organs are influenced by numerous factors such as the season and the weather [20 - 22]. In making this survey, we also took these factors into account as far as this was possible in an outdoor survey.

As for the subjective state of health of the students, we surveyed the subjective symptoms, the past and present history, and the state of health of other family members. When the results were compared for both schools, both the male and the female students of the polluted school generally had higher percentages of complaints than the students of the control school. In particular, symptoms of nasal catarrh, such as clogging of the nose and cold in the nose, as well

as symptoms of rubescence and swelling of the throat, were remarkably frequent. However, in both schools there was not a single case of chronic bronchitis, as defined in the BMRC instruction manual [9]. In view of these facts, one receives the impression that the effects on the human organism manifest themselves here somewhat differently from other cities where air pollution presents problems. No doubt this is because of differences in the quality and quantity of the pollutants. On the other hand, no significant relationships could be found between the length of residence and the students' subjective symptoms, their past and present history, and the state of health of their family members. Almost no significant relationships could be discovered between the distance between their residences and the plant and their subjective symptoms. However, in those students who lived in places less than 0.5 km from the plant, there was observed a tendency for there to be high percentages of throat symptoms during the summer and past history of pneumonia. These two items both appeared with a higher percentage in the polluted school than in the control school. When this fact is taken into consideration, it is supposed that these items have a connection with the pollution discharged from this plant.

There are a number of reports indicating that pneumonia occurs frequently among operatives working with manganese [16, 17]. There are also reports stating that pneumonia is frequently found among the populace residing in the vicinity of manganese plants [16, 17]. Suzuki [16] also reports that past histories of pneumonia are often found in schoolchildren and in the general population in the vicinity of plants handling manganese; he considers manganese as the cause. Our survey may be said to have produced results which are not contradictory to these findings.

As the lung ventilation functions, we measured the forced expiratory volume, the one-second capacity, the one-second ratio, and the maximum expiratory flow. Since these items are influenced by the stature, in these tests we also made studies of the body heights and body weights of the students of the polluted school and the control school. However, the only differences were that the male second

graders in the control school had somewhat greater body height, and the female first graders in the polluted school had somewhat greater body height. In the other grades, no clear differences could be observed between the two schools. Consequently, in our observations of the ventilation functions, we did not take the effects of stature into special consideration.

The mean values of the forced expiratory volume were lower in the polluted school than in the control school for both males and females and for all grades. In the one-second capacity, the one-second ratio, and the maximum expiratory flow, which are used as general indexes of the pulmonary ventilation functions, all of the mean values were lower in the polluted school.

Thus, it was clearly established that the students of the polluted school, located in an area polluted by manganese, had lower pulmonary ventilation functions than those in the control school, which was not affected by manganese pollution. In order to determine whether this is caused by the influence of air pollution emanating from a single, specific source, one must study the relationship to the source of pollution and the relationship to the exposure. For this purpose, one must first study the relationship to the residence. In this survey, one must also take into consideration the fact that the polluted school is located quite close to the source of pollution, being about 100 meters away from the source plant. One must also consider the fact that the tests of the ventilation functions were conducted at the polluted school, which is close to the source of pollution. Among the ventilation functions tested, the one-second ratio is considered to be relatively little affected by the stature. In addition, in this survey there were no differences from grade to grade in the control school. For this reason, it was decided to use the one-second ratio as an index for studying the relationship between the aforementioned factors and the ventilation functions. When the one-second ratios of the students of the polluted school were studied according to their places of residence and their lengths of residence, it was observed that those who had been living in the polluted area for longer than three years had lower one-second ratios than

those who had been living there for less than three years. It was also observed that, among those who had been living in the area for longer than three years, there was a greater drop in the one-second ratio in those students who lived nearer to the plant. Consequently, one can say that those who had lived for a longer time in the polluted area, and those who were living closer to the source of pollution, were more powerfully influenced in their ventilation functions. According to the observations of Suzuki et al [2, 3], the manganese pollution in this area was more pronounced in the areas closer to the plant which was the source of pollution. Therefore, the fact that the ventilation functions declined in those living closer to the plant indicates that the decline in the ventilation functions is caused by pollution emanating from the plant, and that a quantity-reaction relationship exists between the degree of manganese pollution of the atmosphere and the decline in the ventilation functions.

It is clear, both from the reports of Suzuki et al. [2, 3] and from the survey made by the Ishikawa Prefectural Research Institute for Sanitation and Environmental Pollution [1], that pollution by manganese is so slight as to be almost unobservable at a distance of more than 1.5 km away from the plant which is the source of pollution. In spite of this, even among those students of the polluted school whose places of residence were at a distance of more than 1.5 km away from the plant, the one-second ratios had clearly declined in comparison with the students of the control school. It is conceivable that the environmental pollution indexes may not indicate the degree of pollution to an adequate degree. Especially in places at a distance from the source of pollution, these measured indexes may not have a thoroughly satisfactory acuteness. However, in this case more significance ought to be attached to the fact that the polluted school studied in this survey was too close to the source of pollution, that is, the fact that it was located in a place where it was highly subject to the effects of the pollution. Even though their residences may be subjected to almost no effects of the pollution, these students spend most of the day in a place where there is the most intense pollution. That is, one can observe that their school

life in a highly polluted atmosphere no doubt was the chief cause for the decline in the one-second ratio of the studerts whose residences were in areas with almost no pollution.

When the one-second ratios in the different grades in the polluted school are examined from this viewpoint, one finds that the average is 85.4% for the first grade males. On the other hand, the averages for the second and third grade males are lower, being 83.1% and 83.5%, respectively. The same results are obtained for the females. In the first grade females, the average is 86.7%, but in the second and third graders there are lower values of 84.5% and 83.8%, respectively. No differences between the different grades could be observed for the one-second ratios in the control school. Therefore, one may consider that these differences indicate the relationship between the length of schooling in the polluted school and the ventilation functions. At the time when this survey was made, the first graders had been attending school at the polluted school for a period of only three months. For this reason, the first graders had been exposed to less pollution in their school life, and the decline in this functions was also less.

As for the relationship between the degree of pollution of the regional environment and the pulmonary ventilation functions, we were able to establish this clearly from the manganese concentrations in the cryptomeria leaves and in the gutter dirt, as reported by Suzuki et al. [2, 3], and by analyzing the one-second ratios obtained in this survey from the standpoint of the distance away from the plant. That is, as is shown in Figures 5 and 6, there is a quantity-reaction relationship between the manganese concentrations in the cryptomeria leaves and in the gutter dirt and the pulmonary ventilation functions. The results indicate that these pollution indexes can be effective in determining the effects of this type of air pollution on the human organism. In the results depicted in Figures 5 and 6, it is clear that the students of the polluted school who live at a distance away from the plant in areas where the manganese concentration, used as a pollution index for the area, has hardly increased at all still had

one-second ratios lower than those of the students of the control school. The chief cause of this may be sought in the above-mentioned school environment.

The decline in the one-second ratio manifests itself to a greater degree in the females. Concerning this point, it will be necessary to consider the question of sex differences. Especially in this survey, the fact that the tests of the ventilation functions were carried out inside the school, which was subject to intense pollution, may possibly have exerted subtle influences on the results. It has been reported that in tests of ventilation functions of this type, the measured values for females are not connected as closely with pollution as are the measured values for males [19]. There is an undeniable possibility that in these tests, which require forced expiration, the one-second ratios of the females may have been powerfully influenced by psychological factors.

As was described above, it was established clearly that the increases in subjective symptoms and the declines in the pulmonary ventilation functions seen in the polluted school are caused by air pollution due to the plant which discharges manganese. Furthermore, since the school is located close to the plant which is the source of pollution, it was clearly established that the students were also subjected to the influence of the school environment. If the manganese concentrations in the cryptomeria leaves and in the gutter dirt are used as indexes, a quantity-reaction relationship exists between the pulmonary ventilation functions of the students and the degree of air pollution. It is not related to pollution indexes such as the amount of dust fall or the sulfur oxide concentrations. Besides, the concentrations of these substances in the atmosphere were not high enough to have any influence on the ventilation functions.

Manganese is not the only substance polluting the area surveyed. Other metals are also discharged from this plant. However, within the range of the observations, none of the other substances differ from the other areas so clearly as the manganese. Pneumonia is

mentioned as one of the effects of manganese dust on the human organism. One cannot ignore the fact that past histories of pneumonia were significantly numerous among the students of the polluted school.

In sum, one can suppose that manganese dust was the chief cause of the effects on the human organism observed in this survey. Of course, since metals other than manganese are also being melted in this plant, their effects cannot be ignored. However, as of the present time, none of them have yet been observed to be of sufficient importance for the chief cause to be attributed to them.

## VIII. Summary and Conclusion

A survey was made of junior high school students in an area in Kanazawa city which has air pollution caused by N industrial plant, which engages chiefly in the production of ferromanganese. The effects on their respiratory functions were surveyed.

There is conspicuous manganese pollution in this area, but the dust fall and sulfur oxide concentrations are not great enough to present any problem. They have approximately the same values as in the rest of Kanazawa city. A questionnaire survey was carried out, using the BMRC questions, with a few additional questions, and tests were made of the pulmonary ventilation functions, using Tatebe recording Vitaler Meters. The results obtained were the following.

1. In the survey of subjective symptoms, the students in the polluted school had a greater prevalence of sputum, nose and throat symptoms, and past history of pneumonia, than the students in the control school.

2. In the tests of the ventilation functions, the forced expiratory volume, the one-second capacity, the one-second ratio, and the maximum expiratory flow were lower in the students of the polluted school than in the students of the control school. This indicated that they were influenced by the pollution.

3. The one-second ratio of the students of the polluted school was lower the longer had the students been living in the polluted area and the nearer their homes were to the plant. Furthermore, the decline in the one-second ratio was greater in the students who had been attending the polluted school for a longer period of time (the upper grades). This indicated that the students were influenced both by the school atmosphere and by their home atmosphere.

4. An obvious correlation was observed between the manganese content in the cryptomeria leaves and the gutter dirt in the vicinity of the plant and the one-second ratio. It was determined that the manganese concentrations in cryptomeria leaves and gutter dirt can be a good index for the effects of this type of pollution on the human organism.

In view of these points, the conclusion was reached that the increases in the subjective symptoms and the declines in the pulmonary ventilation functions seen in the students of the polluted school were caused by the air pollutants discharged from the N plant, and that these effects on the human organism can be explained without any difficulty in terms of manganese dust.

In conclusion, we express our profound gratitude to the Ishikawa Prefectural Research Institute for Sanitation and Environmental Pollution, the Kanazawa Municipal Board of Education, the school authorities, and the personnel of the Kanazawa Municipal Environmental Pollution Center, all of whom collaborated in this survey, as well as to Dr. Takeo Suzuki of the National Institute of Public Health, who kindly provided us with materials.

A resume of this paper was made public at the 41st convention of the Japan Hygiene Society.

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ATTACHMENT C-12

*Studies of the Effects on the Respiratory Organs of Air Pollution Consisting of Dusts Composed Mainly of Manganese. (First Report) Effects on the Respiratory Organs of Junior High School Students*

Nogawa, K.; Kobayashi, E.; Sakamoto, M.; et al.
Japan Journal of Public Health, 20:315-325, June 1973

Review by Emanuel Landau, Ph.D.

This cross-sectional epidemiological study, carried out in Kanazawa city, Japan, was designed to compare the morbidity experience as well as pulmonary function values for the population of a junior high school exposed to manganese dust from a plant, "N", producing chiefly ferromanganese with a junior high school population not so exposed.

The polluted school, consisting of 1258 first, second, and third junior high school grades, was located approximately 100 meters away from the plant. The control school consisted of 648 students and was separated from the plant by the downtown part of Kanazawa city, a distance of approximately 7 km. Students at the exposed school lived closer to the plant and were therefore also exposed in the non-school environment. The authors summarize their study by stating "the conclusion was reached that the increases in the subjective symptoms and the declines in the pulmonary ventilation functions seen in the students of the polluted school were caused by the air pollutants discharged from the N plant, and that these effects on the human organism can be explained without any difficulty in terms of manganese dust."

Although the data may be compatible with the investigators' interpretations of the effect of manganese on children's respiratory ailments and lung function, the failure to pay attention to sound epidemiological procedures and the inconsistencies and anomalies in the results are evidence of the inaccuracy and inappropriateness of the authors' assessment.

This survey was designed to take advantage of the children as having special advantages as subjects for the study of toxic substances. They are much more unlikely to smoke cigarettes, they don't have occupational exposures to toxicants, their residential history tends to be simpler and, very importantly, their respiratory systems seem to be more sensitive to insult than those of adults, making it easier to detect adverse effects. The authors intended to demonstrate that there was an effect of manganese on the health of the exposed students. Apparently, the investigators believed that the morbidity experience of the exposed school children might serve as a precursor to the pneumonia occurring "frequently among operatives working with manganese" as well as the pneumonia "frequently found among the population residing in the vicinity of manganese plants."

The measurements of ambient manganese were taken at 3 locations at distances within 200-400 meters from the plant and at 4 other stations within the city. At these monitoring stations, continuous measurements are made of the dust fall, the manganese contained in the dust fall, and the sulfur oxide concentration. According to the report, the dust fall in the vicinity of the plant is "somewhat greater than in the other areas, but there is no great difference." However, the level of manganese near the plant is 200 $kg/km^2$/month, 20 times the 8 $kg/km^2$/month away from the vicinity of the plant. Although the sulfur oxide concentrations have been increasing over the years, the difference between near and away from the plant is small.

Unfortunately, the authors failed to take account of known and potential covarying and confounding factors, and these factors have not been adequately controlled or taken into account in the analysis. Known conditions such as socio-economic factors that were present at the time of the health survey were not considered. The availability and adequacy of health care was similarly ignored. Information on home heating was not available, the occupational exposure of the father was not analyzed, the possibility of indoor pollution was overlooked. Family crowding, so important in respiratory illness, was not taken into account. In short, this is another instance of an inability to recognize the importance of obtaining and analyzing the requisite information in addition to exposure to the toxicant

of interest, particularly for respiratory disease. This inability to "control" for socio-economic factors and the other possibly significant variables severely limit the conclusions regarding manganese exposure which can be responsibly drawn from this flawed study.

The authors note the very high (unbelievably high) response rate for the survey and lung function tests in Table 1. This is most impressive. There appears to be a minor arithmetic error in the number enrolled in the control school (645 rather than 648) and in the responses to the questionnaire survey (631 rather than 640).

The British Medical Research Council's Questionnaire (1966 version) was the basis for selection of questions "concerning subjective abnormalities in the eyes and throat" to which "items concerning the health of the other family members were added." One also wonders if an approved and tested Japanese version of the British MRC questionnaire was available for use of the study population.

The tests of "ventilation functions" were performed on three Tatebe recording Vitalor Meters. The reproducibility of a given instrument is not made known to the reader. Also, what is the inter-technician (inspector) variability? The lung function tests included "the forced expiratory volume, the one-second capacity, the one-second ratio, and the maximum expiratory flow" and were measured "by the identical methods, and by the same inspectors." However, the tests were <u>not blinded</u>; the polluted school population was tested for a five-day period beginning July 13, 1970, whereas the control school was tested over a three-day period beginning July 22, Since there is a likelihood that tests which require forced expiration can be influenced by the technician operating the machine, the failure to "blind" is especially disconcerting. The use of the "same inspectors" does not insure the absence of an unintentional bias. It has been repeatedly stated that any error which occurs, or may occur, systematically in one group would bias the results and invalidate the study.

The investigators appear to have a naive view of the difficulty of testing school children in a meaningful fashion. Respiratory function testing in students is a procedure which requires establishment of motivation in the subject. The description of the study design does not indicate how the investigators have controlled for variation in the test team. It is unclear how many inspectors did the testing and how they assured that the subject was performing to maximum capacity. The authors did not discuss how they controlled time per test and other factors at each school to assure the reader of the comparability of testing at all sites. There is no record that children were checked for hay fever or allergies which could influence their ability to perform, nor were they checked for infection of the lungs or asthma. It would be important to know whether infections and allergies influenced the respiratory function testing. If so, then a decrease in pulmonary function by area may represent a problem of active infection rather than chronic lung disease. The problem of active infections in children may result from differences in socio-economic status, mentioned previously, or in home environments rather than manganese pollution. These factors are related to infections which are closely related to family conditions and socio-economic factors.

The report itself notes the inconsistent testing results. It states (Page 27 of the translation): "There is an undeniable possibility that in these tests, which require forced expiration, the one-second ratios of the females may have been powerfully influenced by psychological factors." Again, "the tests of the ventilation functions were carried out inside the school, which were subject to intense pollution, may possibly have exerted subtle influences on the results."

It is regrettable that the investigators were apparently not able to obtain an unbiased set of lung function values. One wonders also if the study was designed to eliminate any systematic differences between operators. The article fails to disclose any recognition of such possible variability.

If the translation is correct, the validation of the questionnaire is problematic, at best. What does it actually mean to "confirm" the entries for each of the items "by interviews during the examinations"? How many staff members were available for the 200-plus questionnaires each of the test days? From extensive experience with surveys, I have learned that confirmation of "soft" symptoms is very difficult even with adult respondents. How much less likely is it to get reproducible replies from teenagers involving illnesses a long time ago? How valid are the completed items regarding the illnesses of their parents during the course of a year? When responses are based on "soft" outcomes, the student's desire to please and his knowledge of environmental conditions may bias results. Observational studies without objective outcomes and with unknown factors which may have influenced the responses must be subject to question. An examination of the subjective symptoms clearly demonstrates that except for possibly pneumonia, pleurisy, and tuberculosis, all of the reported symptoms are highly subjective and what epidemiologists term "soft", thereby indicating the likely lack of reliability of the answers. Incidentally, the excess of TB man confirm the possible difference in socio-economic status, in crowding, etc. The late Professor Donald Reid, in writing of the British air pollution problem before the start of this study, had pointed out that the poorest persons lived in the worst housing in the most polluted parts of London and other British cities. Poverty and pollution were intertwined. Is it poverty and poor medical care which distinguished the students from the polluted school as compared with the "control" school? The failure to address this issue is extremely disconcerting and directly impinges on the soundness of the study.

The study results are interesting but the results are not coherent and consistent. The prevalence of symptoms differs markedly for male and female junior high school students by distance from the plant. There rarely is the expected gradient, by residential distance from the plant, if the level of manganese were clearly associated with the symptom prevalence. Moreover, epidemiologists and statisticians in the United States and England have been taught that "association is <u>not</u> causation." The conclusion that the increase in the symptoms and the decline in lung function "were <u>caused</u> by the air pollutants discharged

from the N plant" is wholly unacceptable as a scientific statement. (underlining added) It is regrettable that enthusiasm has taken the place of science.

The analysis is badly flawed. There is no indication of whether the mean or maximum of several trials in the same subject should be recorded and how may trials of the expiratory measures should be allowed. This is important. There is no indication that this was addressed in the study. Moreover, there is general agreement that since maximum expiratory flow rates vary among individual subjects, prediction equations must be based at least on sex, age, body size, and height. Differences in height were noted and then disregarded since these differences were not "pronounced." It is unlikely that the reader would agree that mean values should be compared without regard to such factors if only small differences are expected in the lung function values.

The analysis also partakes of a rationalization. Since no ambient manganese exposure took place more than 1.5 km away from the plant for students in the polluted school, a decline in the one-second ratios had to be due to their "school life in a highly polluted atmosphere." At the same time, the report notes that students of the polluted school who had been living in the polluted area for less than three years were less affected than those living in the area for longer than three years. Did each year pose a greater risk? Didn't these students attend school during this three-year period? Isn't there an elementary school in the polluted area? Incidentally, was account taken of possible migration through a residence history? The search to demonstrate the presumed influence of manganese is not persuasive.

In summary, this report represents a misinterpretation and overinterpretation of a cross-sectional study whose soundness is seriously open to question. The possible biases, the lack of the necessary knowledge regarding vital co-factors and the lack of coherence and consistency in the results renders a judgment that the stated results "were caused by manganese dust" is without a scientific foundation.

ATTACHMENT C-13

Comments On:

*Studies of the Effect on Respiratory Organs of Air Pollution Composed Mainly of Manganese (First Report) Effects on the Respiratory Organs*. By Nogawa et al. Japan J. Public Health 20:315-325, 1973.

By Ian Higgins, M.D.

A comparison of respiratory symptoms and ventilatory lung function of students attending a junior high school (polluted school) situated 100 meters from a ferro-manganese plant with students at a junior high school (control school) located 7 kilometers away was made. There was conspicuous manganese pollution in the vicinity of the plant, but other pollutants such as dust fall and sulfur oxides are said not to present any problem. Manganese pollution in the air more than 1.5 kilometers from the plant is negligible.

Self-completed British Medical Research Council Questionnaires were obtained and the forced vital capacity (FVC) and forced expiratory volume ($FEV_1$) were measured in over 97% of the students. The questionnaires were confirmed by interviews during the examinations. A higher prevalence of symptoms and lower than average lung function was reported for both boys and girls at the polluted school. Differences were consistent for each grade. The authors noted that the FEV/FVC ratio of the students at the polluted school were lower the longer they lived in the polluted area and the nearer their homes were to the plant. There was an obvious correlation between the FEV/FVC ratio and the manganese content of cryptomeria leaves and gutter dirt, which they suggest is a good index of manganese pollution. They conclude that manganese pollution was responsible for the effects observed.

In a study of this kind, it is important to exclude other differences than pollution between the two groups of children. It is remarkable that nowhere in the paper is the age of the children mentioned. Age and grade are not necessarily synonymous

and could differ between the two schools. Mean height and weight, which may influence lung function, are included in Table 3 but there is no information on how they were measured. Were they measured during the survey? Or were they extracted from school records? With the exception of the second graders, height does not appear to account for much of the lung function differences.

No mention is made of socio-economic circumstances. Persons who live in polluted areas are usually poorer and in worse health than persons who live in less polluted neighborhoods. Table 2 suggests that family members had a higher prevalence of respiratory diseases in the polluted school than in the control school. We do not know, however, if this information was given by the student or confirmed by the family members. It probably has little validity. The same reservation may apply to the apparently higher TB rates in the polluted school. This too, might indicate a social difference between the two groups of children.

Nothing is said about smoking, except that "there are no effects of smoking in junior high school students" by which I think they mean they do not smoke. This is not true; there could be smokers in children of this age. Information on smoking habits of family members does not seem to have been collected. Heavier smoking of parents and children in the polluted school could have caused the differences in symptom prevalence and lung function.

Any serious comparison of pollutant concentrations in the two groups of children seems to have been perfunctory. There is said to have been little difference in the total suspended particles and sulfur oxides in the two areas. However, Table 5 shows that the TSP 100 meters from the school was approximately 300 $mg/m^3$. Presumably it could have been much higher than this on some occasions.

All in all, while respiratory findings can be attributed to the pollution from the plant and possibly to manganese, other, more likely explanations cannot be excluded.

# ATTACHMENT C-14

Bleecker (1988)

Neurotoxicology and Teratology, Vol. 10, pp. 475–478, Pergamon Press plc, 1988. Printed in the U.S.A. 0892-0362/88 $3.00 + .00

# Parkinsonism: A Clinical Marker of Exposure to Neurotoxins

MARGIT L. BLEECKER[1]

*Johns Hopkins University School of Medicine, Department of Neurology*
*Francis Scott Key Medical Center, Baltimore, MD 21224*
*Johns Hopkins University School of Hygiene and Public Health*
*Department of Environmental Health Sciences, Division of Occupational Medicine, Baltimore, MD 21205*

BLEECKER, M. L. *Parkinsonism: A clinical marker of exposure to neurotoxins*. NEUROTOXICOL TERATOL 10(5) 475–478, 1988.—Parkinsonism must be viewed as a final common pathway resulting from a variety of neuropathological lesions which interfere with the integrity of the nigrostriatal system or its output. Exposure to a wide variety of neurotoxic compounds, namely, carbon monoxide, carbon disulfide, manganese and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), are associated with parkinsonism that has a varying neuropathology, which will be discussed.

Parkinsonism  Neurotoxins  Nigrostriatal system

FOLLOWING exposure to a wide array of neurotoxic compounds, namely, carbon monoxide, carbon disulfide, manganese and MPTP, parkinsonism may develop. What does this clinical constellation of physical signs, namely, rigidity, tremor, akinesia and loss of postural reflexes tell us about the underlying mechanism? Since most, if not all, of the motor features of Parkinson's disease are due to the loss of dopaminergic cells in the substantia nigra (16,26) can it automatically be assumed that this is the site of action for these neurotoxic compounds? Parkinsonism must be viewed as a final common pathway resulting from a variety of neuropathological lesions which interfere with the integrity of the nigrostriatal system or its output. In contrast, the cardinal feature of Parkinson's disease neuropathology is loss of cells in the substantia nigra (18) and degenerative changes in other pigmented nuclei in the brainstem, particularly the locus ceruleus. Many neuropathologists require the presence of Lewy bodies, intraneuronal eosinophilic inclusions, to confirm the diagnosis of Parkinson's disease.

## CARBON MONOXIDE

The neurological manifestations of acute CO intoxication are well documented in a vast literature (22). As a consequence of brain hypoxia or anoxia, the most common neurological abnormality described in acute CO poisoning is a disturbance of consciousness. Of growing concern and considerable clinical interest is the syndrome of delayed encephalopathy following acute CO poisoning. The onset of delayed neurologic deterioration occurs following a free interval of 2–60 days, during which the patient appears to have apparently recovered. Mental abnormalities, cortical dysfunction, parkinsonism and pyramidal lesions may often develop abruptly (21,61). CO-induced parkinsonism can occur as an isolated pure syndrome (33), but much more frequently it occurs in the context of diffuse brain damage, with a variety of other neuropsychiatric sequelae (52).

The reported prevalence of delayed encephalopathy following acute CO intoxication varies between 0.06–18.5% (11, 22, 40). A recent epidemiological study from Korea (11) showed that delayed neurological deterioration occurred in 65 from among 2,360 CO intoxicated patients examined, being 11.8% of those requiring hospitalization. Systemic factors associated with age and initial deep coma favored this complication. Three quarters of the patients recovered within one year, though some showed presistent mild memory failure or parkinsonism (11,21).

Neuropathological studies (7, 23, 28, 57) and computerized axial tomography (CAT) findings (11, 27, 31, 33, 45, 48, 54, 56) in cases of acute and delayed CO encephalopathy with and without parkinsonism demonstrate bilateral basal ganglia necrosis with a predilection for involvement of the globus pallidi. The globus pallidus lesions usually affect the anterior two thirds of the pallidum and often extend into the adjacent white matter of the internal capsule (23). The involvement of the pallidum is thought to be due to the endarterial vascular supply to this structure, predisposing it to regional hypoperfusion in conjunction with systemic hypotension, anoxia, and metabolic acidosis caused by CO (24).

Other gray matter structures which may be involved in addition to the pallidum, include caudate, putamen, substantia nigra, hippocampus, thalamus, hypothalamus, cerebral cortex, cerebellar Purkinje cells, and dentate nuclei. CO intoxication may also result in a multifocal or diffuse leukoencephalopathy with demyelination and subsequent necrosis of the centrum semiovale, periventricular regions, and fiber tracts connecting the cerebral hemispheres (7, 23, 38).

Some cases in which CO has produced large bilateral globus pallidus lesions, a full remission of parkinsonism following L-dopa treatment has been shown. Enough efferent output may have been preserved in the pallidum for augmentation of striatal dopamine levels with L-dopa to have a beneficial effect (28).

However, Klawans *et al.* (32,33) postulate that parkinsonism, secondary to intrinsic striatal neurochemical dysfunction or structural damage, may not be associated with depletion of nigrostriatal dopamine levels or with beneficial response to dopamine precursors. This may explain why many cases of CO-induced parkinsonism have not responded to L-Dopa compounds.

## MANGANESE

Historically, manganese intoxication was first observed in brownstone millers and in workers involved in mining and processing manganese ores, who inhaled toxic amounts of manganese dust (14). Chronic manganism produces an irreversible syndrome which bears a striking resemblance to Parkinson's disease (13). The symptoms include fixed gaze, bradykinesia, postural difficulties, rigidity, tremor and dystonia. It is the dystonia, in addition to the often prominent mental status changes, which separate parkinsonism due to chronic manganese intoxication from Parkinson's disease (4).

At the neuropathologic level the two conditions appear to diverge even further. As would be expected, the core feature of involvement of the basal ganglia is indeed found, accounting for the clinical features of parkinsonism. However, autopsy reports (2, 10, 17, 30, 46, 55, 60) have shown the most extensive changes to be in the striatum pallidum, areas which are spared in Parkinson's disease. Shrinking of the basal ganglia, together with marked degeneration of nerve cells, and the presence of gliosis, are noted in these areas. Substantia nigra degeneration, although a less prominent feature of manganese toxicity, has been observed to a variable degree.

Although the neurotransmitter effects of manganese have been documented primarily in animal studies, it is of note that the only neurochemical report in a case of human manganese toxicity did show a depletion of striatal dopamine (6). Norepinephrine was also lowered in the hypothalamus, while serotonin levels were normal. Lewy bodies have never been observed in manganese-induced parkinsonism.

L-Dopa treatment seemed appropriate in patients with manganese-induced parkinsonism, but results have been somewhat contradictory. Six patients treated with L-Dopa by Mena and colleagues (44), showed improvement in rigidity, hypokinesia, and postural orientation. Rosenstock and colleagues (53) also reported a therapeutic response in one paitent. These results have not been confirmed by others (12,15), although the possibility that there are two clinical subgroups of patients was suggested as an explanation.

The neuropathologic features of Parkinson's disease and manganese-induced parkinsonism differ diametrically, one affecting the input side of the basal ganglia (i.e., the substantia nigra in Parkinson's disease) and the other its output side (i.e., the striatum and globus pallidus in manganese toxicity).

Manganese toxicity more closely resembles another disease which causes parkinsonism known as striatonigral degeneration. In fact, elevated manganese levels have been found in this condition (8). In summary, the major effects of manganese toxicity are found in the cells of the striatum and globus pallidus which are not dopaminergic.

## CARBON DISULFIDE

Around the turn of the century, the viscose rayon industry was established, and the production of rayon from wood pulp, a process involving the use of large amounts of carbon disulfide, brought with it the recognition that chronic poisoning with carbon disulfide produced parkinsonism. It was in this setting that Quarelli (49) observed varying degrees of parkinsonism in 30% of viscose rayon workers in Torino. In a study of workers in the American rayon indsutry 21.7% appeared to have experienced some type of adverse affect; 1 in 6 of these individuals evidenced an extrapyramidal syndrome (39). In this study the criteria for parkinsonism included the appearance of rigidity, the absence of arm swinging when walking and loss of postural reflexes. A recent report has cited a high incidence of parkinsonism in agricultural workers involved in the storage of grain, and suggested that $CS_2$ may play a role in this syndrome (47).

In regard to the neuropathological effects of $CS_2$ poisoning in humans, surprisingly little is known. In the single study of $CS_2$ exposures of nonhuman primates, four rhesus monkeys were exposed, by inhalation, to 50 ml/6 hr daily for 12 to 21 months (51). Symptoms of plastic, cogwheel rigidity, bradykinesia, postural freezing and tremor said to closely resemble Parkinson's disease were observed. The most striking and consistent pathological finding in these animals was a pronounced pallidonigral degeneration.

Although degenerative changes within the basal ganglia have been reported in dogs (1) and mice (34) exposed to $CS_2$, consistent or uniform involvement of these structures have not been observed, for the most part, in other species (5). As was true with manganese, the output structures of the basal ganglia (i.e., the striatum and globus pallidus) rather than the substantia nigra, are predominantly affected.

Biochemical studies in rats have not shown dopamine depletion as a consequence of $CS_2$ exposure, although a depletion of CNS norepinephrine (NA) has been reported (41). Interestingly, animals studied after only two days of exposure showed a 16% elevation of striatal dopamine. These findings can probably both be explained by the subsequent observation that metabolites of $CS_2$ inhibit dopamine-beta-hydroxylase (41).

$CS_2$ damage to basal ganglia structures may arise out of the inhibition of dopamine $\beta$-hydroxylase which could leave the cell exposed to the potentially damaging effects of superoxide. Another mechanism which as been proposed for the toxic effects of $CS_2$ involves the formation of covalent adducts with pyridoxamine (59). The consequent derangement of $B_{16}$ metabolism could be responsible for the neurotoxicity of $CS_2$ and supplementation of the diet with $B_6$ has delayed the onset of some of the neurotoxic effects of $CS_2$ in rats (58) although it is still not clear why this would predispose basal ganglia structures to damage.

## MPTP

The first and most striking MPTP toxicity in humans is

that it produces an unalloyed parkinsonian state (3). Not only does MPTP produce all of the major features of Parkinson's disease, but many of the more subtle features of the disease, such as kinesia paradoxica and seborrhea are present as well.

In regard to response to treatment, the parallel continues, as humans with MPTP-induced parkinsonism respond to the full array of antiparkinsonian agents in a manner quite analogous to that seen in Parkinson's disease (36). This further distinguishes it from manganese-induced parkinsonism and that caused by carbon disulfide and carbon monoxide. In these conditions the results of therapy are much less clear. Finally, the full array of complications typically seen in idiopathic Parkinson's disease with L-dopa therapy are also encountered in patients with MPTP-induced parkinsonism (36).

The primary neuropathological feature of MPTP neurotoxicity is degeneration of dopaminergic neurons of the substantia nigra (9, 15, 19, 37). This distinguishes it from the other toxins known to cause parkinsonism in man, which have their major effects in the striatum and pallidum rather than in the substantia nigra.

In a series of six squirrel monkeys (of middle to old age) MPTP does also induce degeneration in the locus ceruleus (20). In the same group of older monkeys given MPTP [Forno *et al.* (20)] three animals (all over the age of 15 years, or the equivalent of 60–70 years of age in the human) developed eosinophilic inclusions in the CNS, which were intraneuronal and typically demonstrated a peripheral halo (20). These structures have been seen only in areas where Lewy bodies occur in human parkinsonism (18).

Little is known how MPTP or its metabolites kill neurons but the suggestion that age may be a critical factor in causing neurodegeneration has been frequently speculated (29,50). The toxicity of MPTP depends on its conversion in a two step process to 1-methyl-4-phenylpyridinium ion ($MPP^+$), the first step involving monoamine oxidase (MAO) B. Studies have shown that older rodents are indeed more efficient in converting MPTP to $MPP^+$ (35). These observations are compatible with the suggestion that increasing concentrations of MAO with age may play a role in regard to the enhanced susceptibility of aged animals to MPTP (29). MAO is known to increase with age, presumably by virtue of glial proliferation. With MPTP glia appear to be converting a nontoxic substance into a compound which selectively kills neurons.

In summary, parkinsonism may be a clinical marker of exposure to a neurotoxin but is associated with a variety of neuropathological mechanisms. Also, as mentioned, the aged nervous system provides a substrate which has less regenerative abilities and therefore may expess irreversible pathology earlier and at lower doses. Future research focusing on the interaction between age-related changes in the nervous system and the biologic effects of neurotoxins may prove to be fruitful in defining the underlying mechanisms producing Parkinson disease.

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